A study of near-ultraviolet radiation induced oxidative damage in Escherichia coli

Coombs, Anne-Marie

Award date:
1988

Awarding institution:
University of Bath

A STUDY OF NEAR-ULTRAVIOLET RADIATION INDUCED OXIDATIVE DAMAGE IN *ESCHERICHIA COLI*.

Thesis

Submitted by

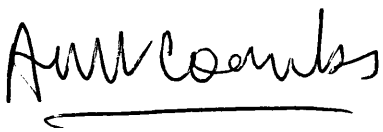
Anne-Marie Coombs, B.Sc.

for the degree of

Doctor of Philosophy of the University of Bath

1988

This research has been carried out in the School of Pharmacy and Pharmacology of the University of Bath under the supervision of S. H. Moss, M.Sc., Ph.D., M.P.S.



ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ACKNOWLEDGEMENTS

I would like to thank Dr. Stephen Moss for the encouragement and helpful guidance he has given me throughout this project.

I should also like to thank Professor John Rees for providing facilities for this research, Dr. David Davies for helpful comment and the Science and Engineering Research Council for providing funding.

My thanks are also due to the technical staff of the Pharmaceutical Microbiology Department who have provided much help in the preparation of experimental equipment, to Mr. David Vinicombe for help with figures and to Jane Higgins for typing the manuscript.

I should like to thank those with whom I have worked throughout this project; Jackie Chamberlain, Aspa Kralli, Sharon Moore, Marcia McAleer, Anthony Smith and Sue Wilson, who have provided encouragement and above all valuable friendship.

Finally, I should like to thank my family and my husband, Richard Jones. Their continued understanding and support was the greatest help of all.

ABSTRACT

Near-ultraviolet radiation is a component of terrestial sunlight. Although mechanisms of cell damage have been identified for far-UV radiation, the mode(s) of action of near-UV radiation are less well defined. One area of current interest is the role of oxidative damage in near-UV radiation lethality. Evidence suggests that cells undergoing near-UV irradiation are exposed to damaging oxygen species. Hydrogen peroxide has been shown to be produced during the near-UV irradiation of cells and as such may have an important role in near-UV radiation damage.

The response of different strains of *Escherichia coli* cells to H_2O_2 and near-UV radiation challenge was examined. It was found that there was a common inducible protective effect for both H_2O_2 and near-UV challenge when cells were pretreated with non-lethal levels of H_2O_2. This induction process also caused an increase in H_2O_2 scavenging ability. However, although there was correlation between H_2O_2 scavenging ability and H_2O_2 sensitivity, a correlation was less apparent with near-UV radiation. The addition of exogenous catalase to the post-irradiation plating medium also protected cells from near-UV radiation damage.

One *E. coli* strain, B/r, was found to be sensitive to near-UV radiation whilst in exponential phase. Growth phase experiments showed that this period of sensitivity was transient and associated with the end of the lag period of growth. Experiments with an isogenic *relA*$^+$/*relA*$^-$ pair of B/r strains showed that in the relaxed strain no such region of sensitivity was seen. The results suggest an involvement of the stringent response in growth phase dependent near-UV radiation sensitivity.

Another *E. coli* strain, SR246 was found to be very sensitive to near-UV radiation under certain pre-irradiation growth conditions. This strain was also found to be sensitive to singlet oxygen when compared to other near-UV radiation resistant strains. Genetic studies were carried out to try to identify the gene(s) involved in the near-UV radiation sensitivity of SR246.

CONTENTS PAGE

INTRODUCTION

PART 1

Ultraviolet light is a natural component of the terrestial environment. Most organisms are exposed to ultraviolet radiation at some point in their life cycle and consequently have evolved mechanisms that allow them both to adapt and make use of the beneficial effects and protect against the damaging effects of ultraviolet light. This thesis is concerned with how the bacterium *Escherichia coli* responds to the near-ultraviolet radiation component of the ultraviolet spectrum and with some of the mechanisms involved in repairing near-ultraviolet radiation induced damage.

Ultraviolet radiation is the radiation beyond the violet end of the visible part of the electromagnetic spectrum and has a range of wavelengths from approximately 190 nm, below which ionization of atoms and molecules occurs, to 400 nm, above which the radiation is detected by the human eye, that is visible light. Wavelengths in the ultraviolet region may be subdivided according to physical and biological properties as follows;

Far-UV	(UVC)	190-290 nm
Mid-UV	(UVB)	290-320 nm
Near-UV	(UVA)	320-400 nm
(visible		400-750 nm).

Solar-UV (290-400 nm), which includes near-UV and mid-UV radiations, has no far-UV component as atmospheric ozone screens out wavelengths below approximately 290 nm. In addition, far-UV and near-UV radiations have different mechanisms of biological action for many important effects such as cell killing and mutation. The mid-UV is a region of transition and of particular interest in dermatology as it appears to have a major role in sunburn and skin cancer in humans. In this respect, mid-UV is

distinguished from near-UV by its effects on human skin, with mid-UV causing erythema whilst near-UV does not.

The relationship between the energy and the wavelength of radiations is given by the expression:

$$E = \frac{h.c}{\lambda}$$

where E = the energy of the quantum in Joules.

h = Planck's constant (6.624×10^{-34} J.sec)

c = velocity of light (3×10^{10} cm.sec^{-1})

λ = wavelength in cm.

Thus the energy of a quantum (or photon) is inversely proportional to the wavelength.

The Effects of Far-UV Radiation on Living Cells

Knowledge of far-UV actions is necessary in order to understand the actions of mid-UV and near-UV radiations. The subject has been treated in detail in many reviews, a recent one being a book by Harm (1980).

Gates (1930) determined that the action spectra for killing of *E. coli* and *Staphylococcus aureus* resembled the absorption spectrum for nucleic acid. The action spectra also showed that there was a peak in the killing of cells, which occurred around 265 nm. These observations introduced the idea of a specific, vital target being involved in inactivation contradicting previous ideas that shorter wavelength (and thus higher energy) radiations caused greater bactericidal action.

Since these early observations, many action spectra have been produced which support the conclusion that DNA is the chromophore for far-UV radiation lethal effects. The nature of the DNA damage induced by far-UV

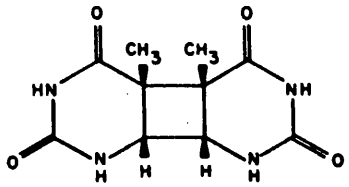
radiation has been widely studied and a variety of photoproducts have been found (fig. 1). The most important of these is the pyrimidine dimer. These dimers are produced between adjacent pyrimidines in the same strand of a DNA molecule. Covalent bonds are created by the two 5-positions and the two 6-positions of the adjacent pyrimidines resulting in a cyclobutane ring. The consequence of this dimerization is the loss of the double bonds at the 5,6 positions of the pyrimidine rings resulting in an altered absorption spectrum. The dimer is a very stable photoproduct although its formation can be reversed by light-dependent and dark-repair systems. Formation of the pyrimidine dimer distorts the component bases producing deformation of the double helix. This lesion interrupts both transcription and replication and is responsible for the majority of the lethal action of far-UV radiation on cells.

Other photoproducts (fig. 1) include: pyrimidine hydrates which are formed in DNA in solution; the spore photoproduct which is produced only in spores or dried cells; pyrimidine adducts which are produced by far-UV radiation at about $^{1}/10$ the rate of dimers but show a new absorption peak at 315 nm. Far-UV irradiation of cells also produces DNA-protein crosslinks but these are only important at high fluences and under conditions that prevent the formation of dimers.

Photorepair

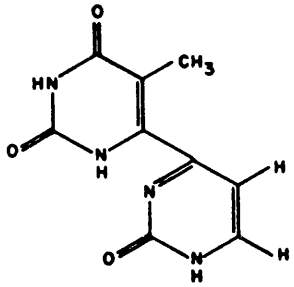
The integrity and survival of the genome depends on DNA being repaired once it has been damaged by UV radiation or other inactivating or mutagenic stresses. There are two broad classes of repair; repair systems that are efficient and relatively error-free, such as photoreactivation and excision repair and the error-prone post-replication repair systems where mistakes made in correctly repairing DNA are one major source of mutation. The

(a) DIMER

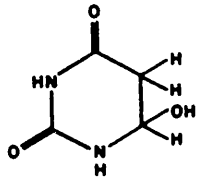


cis-syn

(b) ADDUCT



(c) HYDRATE



(d) SPORE PHOTOPRODUCT

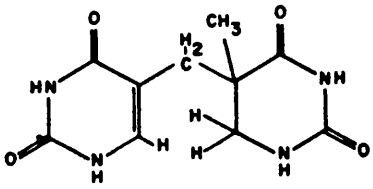


Figure 1: DNA photoproducts found in far-UV irradiated cells.

following paragraphs represent a very brief synopsis of DNA repair. A book by Friedberg (1985) gives an extensive and detailed account of all aspects of DNA repair and thoroughly reviews recent literature in the field.

Photoreactivation

Photoreactivation (PR) is a highly specific mechanism for the repair of UV radiation-induced pyrimidine dimers. It was recognized by Kelner in 1949. Kelner was investigating the effects of UV radiation on the fungus *Streptomyces griseus* when he realised the importance of light in the recovery of UV-irradiated cells. In the same year, Dulbecco (1949) observed this phenomenon in T-group coliphage and called the effect PR. A decade later it was demonstrated that PR is an enzyme-mediated system (Rupert *et al.*, 1958; Rupert, 1960). This PR enzyme, or DNA photolyase, has since been detected in many plant and animal cell extracts (Werbin, 1977).

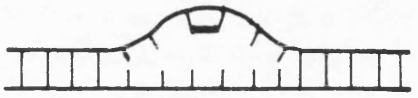
Photoreactivation is a light-dependent process involving the enzyme catalyzed monomerization of pyrimidine dimers. Light of wavelength greater than 300 nm is absorbed by the enzyme and activates monomerization. The enzyme is released after breakage of the dimer cyclobutane ring generating two free, unlinked pyrimidine bases. In *E. coli* the PR enzyme is the product of the *phr* gene. Photoreactivation is illustrated diagrammatically in fig. 2. For a review of PR, see Sutherland, 1977.

Excision Repair

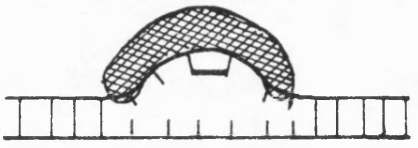
Early evidence for excision repair in bacterial cells arose from the work of Setlow and Carrier (1964) and Boyce and Howard-Flanders (1964) who observed that thymine dimers were removed from DNA of wild-type *E. coli* strains during post-irradiation incubation, but this did not happen in

(1) NATIVE DNA

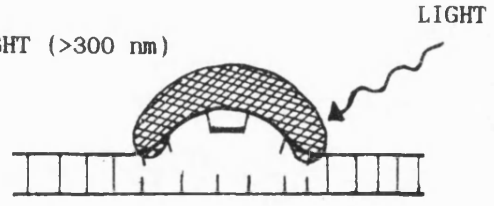
(2) PYRIMIDINE DIMER IN IRRADIATED DNA



(3) COMPLEX OF DNA WITH PR ENZYME (SHADED AREA)



(4) ABSORPTION OF LIGHT (>300 nm)



(5) RELEASE OF ENZYME TO RESTORE NATIVE DNA

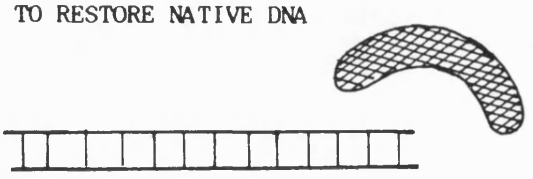


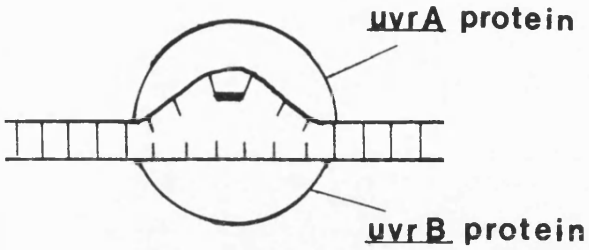
Figure 2: Enzymatic photoreactivation of pyrimidine dimers.

other UV-radiation sensitive *E. coli* strains. The loci involved in this process were designated *uvrA*, *uvrB* and *uvrC*. A sequence of events occurs during excision repair; (a) the uvrABC endonuclease recognises the structural defect caused by a thymine dimer and the products of *uvrA* and *uvrB* bind to the damaged region of the DNA. In the presence of the *uvrC* gene product, the damaged strand is nicked at the 5' end of the damaged region. The endonucleases concerned with this incision event may also be specific for an apurinic or apyrimidinic site i.e. an AP endonuclease. (b) The *uvrC* gene product enters the gap and excises the strand, including the dimer in a 5' --> 3' direction. (c) At the same time and also in the presence of the *uvrC* product, repair replication is initiated by DNA polymerase (coded for by the *polA*, *polB* and *polC* genes). The polymerase binds to the nick and adds, in an 5' --> 3' direction, bases that are complimentary to the intact strand. (d) When the damaged region has been passed, the polymerase introduces a second nick releasing the damaged DNA. The polymerase may continue removing individual bases, thus moving the nick in a 5' --> 3' direction. Finally the gap is sealed by DNA ligase. The process of excision repair is represented in fig. 3.

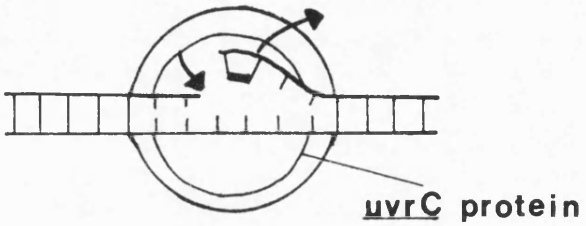
Post-replication Repair

Despite the efficient repair of DNA damage by PR and excision repair, lesions may still be present in the chromosome at the time of DNA replication. These lesions are not recognised during replication as they do not resemble a known base. However, replication does proceed past these lesions leaving a gap in the daughter strand opposite the lesion. Early evidence for this was provided by Rupp and Howard-Flanders (1968). They proposed a model for the subsequent sealing of these gaps which is frequently referred to as post-replication repair. Rupp and Howard-Flanders proposed in their model that once replication had occurred, the

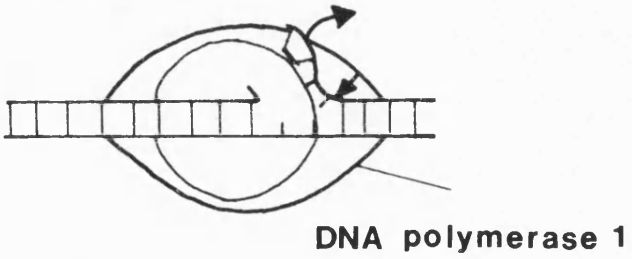
(1) RECOGNITION



(2) INCISION



(3) REPAIR REPLICATION AND EXCISION



(4) LIGATION

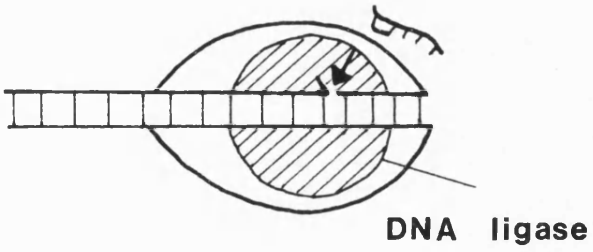


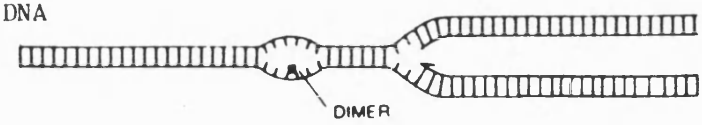
Figure 3: Excision repair

gap opposite the lesion in one daughter DNA duplex was filled by a recombinational event with the complete corresponding region of the other daughter DNA duplex. The resultant gap in the latter was filled using the complementary strand as a template. A diagram representing this process is shown in fig. 4. The mechanism is dependent on the *recA* gene product. For a recent review see Smith *et al.*, 1987.

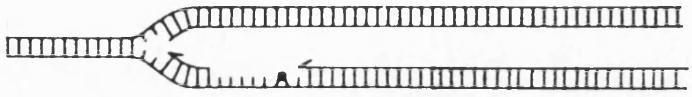
SOS Repair

Another error-prone DNA-repair system is the 'SOS' response. This involves the activation of a number of genes which help protect the cell against genetic damage such as that produced by far-UV radiation, X-rays, thymine starvation, mitomycin C and nalidixic acid. The *lexA* gene produces a repressor molecule that represses the *recA* and other repair genes. The repressor also represses the *lexA* gene itself so that normally there is little LexA protein available. A post-replication gap produced by DNA synthesis opposite a lesion causes RecA protein to attach to this site. This attachement activates the protease activity of the RecA protein which then cleaves the *lexA* gene. This results in a derepression of repair genes (such as *uvrA* and *uvrB*) and of the *lexA* gene. The consequence of this is that a whole spectrum of repair genes are activated to cope with the damage. SOS also affects other bacterial activities besides DNA repair; it stops DNA synthesis, prevents formation of the cell septum during DNA replication leading to filamentation of bacteria and it induces development of prophage λ. The SOS response has been reviewed extensively by Walker (1985) and in shorter reviews by Sedgwick (1986) and Ossanna *et al.* (1987).

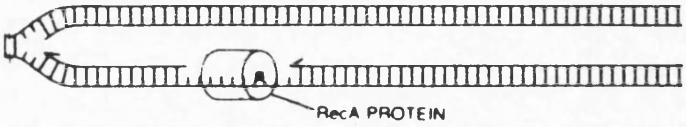
(1) DIMER IN IRRADIATED DNA



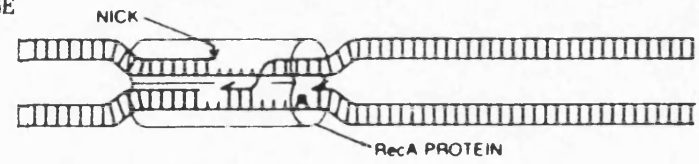
(2) LESION PASSED LEAVING GAP IN DAUGHTER STRAND



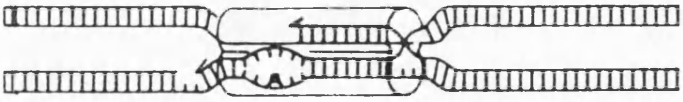
(3) REC A GENE PRODUCT BINDS TO SINGLE STRAND REGION



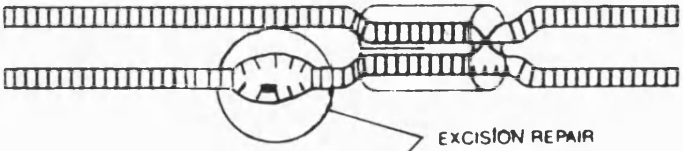
(4) *REC A* NICKS CORRESPONDING STRAND AND PRODUCES RECOMBINATION EVENT AND CROSS STRAND EXCHANGE



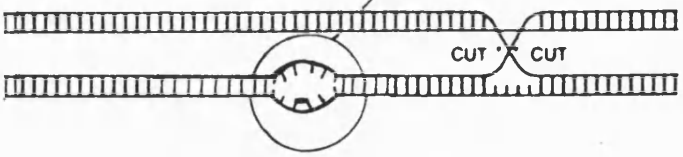
(5) CORRESPONDING STRAND REPAIRED BY DNA POLYMERASE



(6) REC A PROTEIN RELEASED AND DIMER REMOVED BY EXCISION REPAIR ENZYMES



(7) CROSS STRAND EXCHANGE CUT



(8) DIMER CONTAINING REGION RELEASED AND NATIVE DNA RESTORED

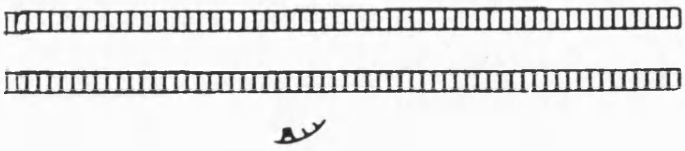


Figure 4: Post replication repair

The Effects of Near-UV Radiation on Living Cells

It is well established that the primary effect of far-UV radiation is to damage DNA and that pyrimidine dimers are the major lethal lesion. These targets and lesions are also seen to be involved with near-UV radiation damage. However, it has become increasingly apparent that other effects play an important part in near-UV radiation damage and that there are marked differences between the modes of action and the types of damage caused by near-UV and far-UV radiations. It is important that the nature of the near-UV radiation effects are elucidated as it is the major ultraviolet component of terrestial sunlight to reach the surface of the earth and, as such, may represent an important environmental stress to many organisms.

Hollaender (1943) published an early report on the effects of long wavelength ultraviolet and short wavelength visible radiations (350-490 nm) on bacteria. He reported that high fluences of near-UV and visible light could kill *E. coli* in the absence of added photosentizer. He also reported that the incident energy required for 50% lethality at 350-490 nm radiation was approximately 10^5 times greater than that required at 265 nm to give the same degree of kill. Dulbecco and Weigle (1952) and Hill (1956) provided further early evidence in support of differences between the effects of long and short wavelength radiations. Dulbecco and Weigle (1952) showed that sublethal exposure to white light diminished the ability of *E. coli* B to support the growth of T2 phage. Hill (1956) confirmed this finding with T1 phage and showed that illuminated bacteria recovered their ability to support phage growth if they were incubated in a nutrient media after illumination but before phage infection.

Since these early findings there have been many reports presenting data on the effects of near-UV radiation on bacteria and other organisms.

The lethal and mutagenic effects of near-UV radiation have been thoroughly reviewed (for example see Webb, 1977 and Jagger, 1985). As a result of these investigations, four fundamental differences between near-UV radiation and far-UV radiation killing have been recognised:

(1) The near-UV radiation-killing of most strains under normal conditions (low fluence rate at room temperature in air) shows no photoreactivation, suggesting that pyrimidine dimers are not the major lethal lesion.

(2) Near-UV killing is more effective in the presence of oxygen, suggesting the action of photodynamic (photo-oxidative) processes. Far-UV killing is not oxygen dependent.

(3) The survival curves that result from near-UV irradiation show more pronounced thresholds or shoulder regions then those that occur after far-UV irradiation. This suggests that repair or recovery occuring at low near-UV radiation fluences is inactivated by high near-UV radiation fluences.

(4) NUV killing is relatively inefficient, the fluences required being 4 to 6 orders of magnitude higher than those required for far-UV killing. As a result, the sublethal effects of ultraviolet radiation, which are overshadowed by the lethal effects in the far-UV region, become more apparent and more important with near-UV radiation.

The Lethal Effects of Near-UV Radiation

Luckiesh (1946) published a preliminary action spectrum for lethality in *E. coli* extending from 200 to 700 nm. This confirmed the earlier report of Hollaender (1943) that high fluences of near-UV radiation and visible light can kill bacteria. Since these original reports, action spectra for

lethality that extend into the near-UV radiation region have been published for a number of organisms. These have been reviewed by Webb (1977). A typical action spectrum for lethality in *E. coli* is shown in fig. 5. This shows the action spectrum for killing of stationary phase *E. coli* WP2s (*uvrA*) irradiated at 25°C under aerobic and anaerobic conditions. Also included in fig. 5 are the absorption spectra for thymidine and for *E. coli* DNA. Several observations can be made from this action spectrum:

(1) The action spectrum for lethality for *E. coli* WP2s closely follows the absorbance of DNA over the range 240-313 nm. At wavelengths longer than 320 nm solutions of pure nucleotides show little absorption and it becomes difficult to measure the absorbance of DNA, although absorption of double stranded DNA has been observed at a low level at wavelengths upto 360 nm by using procedures that subtract contributions due to light scattering (Sutherland and Griffin, 1981). However, Webb (1977) estimated that the absorbance of thymidine at 330 nm was $1x10^{-8}$ of its absorbance at 260 nm and that the sensitivity ratios, in *E. coli* B/r Hcr, based on quantum units, were $1.9x10^{8}$ for 260 nm versus 550 nm and $1.3x10^{9}$ for 260 nm versus 650 nm. This makes it unlikely that direct absorption by DNA can account for the biological effects seen.

(2) The second observation that can be made from the action spectra shown in fig. 5 is that at wavelengths longer than 320 nm cell inactivation is more efficient under aerobic conditions than under anaerobic conditions. Oxygen dependence for lethality has been reported for a number of micro-organisms. For example, a carotenoid-deficient, colourless mutant of *Sarcinia lutea* exhibited oxygen dependent inactivation by sunlight (Mathews and Sistrom, 1959); oxygen dependent inactivation was seen in *E. coli* B/r Hcr with wavelengths greater than 313 nm (Webb and Brown, 1976) and in wild type, *urvA*, *recA* and *polA* *E. coli* K12 mutants at 365 nm (Tyrrell, 1976);

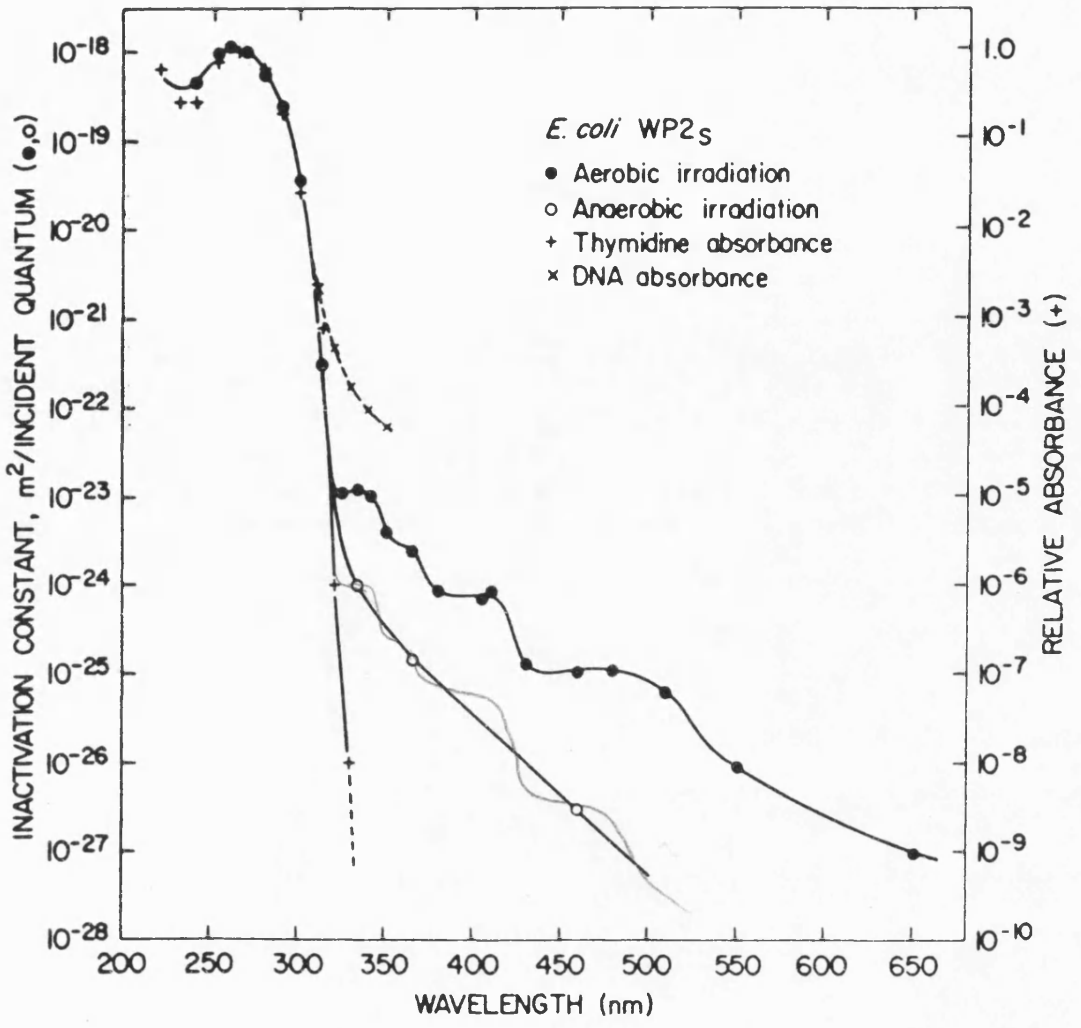


Figure 5: Action spectra for killing of stationary phase *E. coli* WP2s *uvrA* cells irradiated in the presence (●) or absence (○) of oxygen (irradiation at 25°C). Also, the absorption spectra of thymidine (+) and *E. coli* DNA (x). (Adapted from Jagger, 1985.)

broad-band near-UV irradiation of recombination deficient *Salmonella typhimurium* was also shown to be oxygen dependent (Eisenstark, 1970). Inactivation at 254 nm is independent of the presence of oxygen (Webb and Lorenz, 1977) indicating that oxygen dependence is a clear distinction between the biological effects of near-UV and far-UV radiations. This oxygen dependence at wavelengths greater than 320 nm suggests that the lethal effects of near-UV radiation may involve photoproducts produced through photodynamic (photo-oxidative) action.

(3) The third point to be raised about the action spectra for lethality of stationary phase *E. coli* cells (fig. 5) is that in air, significant plateaux, or shoulders, are seen at 340, 365, 410 and 500 nm suggesting absorption by specific chromophores. These will be discussed in the next section.

Targets for Near-UV Radiation Lethality

DNA

Transforming DNA has been shown to be inactivated by 365 nm radiation (Peak *et al.*, 1973) and by wavelengths upto 460 nm (Cabrera-Juárez *et al.*, 1976) which indicates that DNA can be the target for near-UV radiation lethality. As discussed above, the mechanisms of damage and the types of damage produced, may be different in the near-UV region from those in the far-UV region. However, the likelihood that DNA is a principal target for near-UV radiation inactivation is supported by several lines of evidence. Firstly, agents such as oxygen alter not only the sensitivity of transforming DNA to near-UV radiation, but also alter cell survival in a comparable way (Peak *et al.*, 1973). Secondly, DNA repair has been shown to play a major role in near-UV radiation killing in *E. coli*. Eisenstark (1970) reported differing sensitivities between recombination deficient

strains of *S. typhimurium* and *E. coli* and their parent, repair-competent strains. Webb and Brown (1976) showed *uvrA* and *recA* strains of *E. coli* to be slightly more sensitive to near-UV radiation (365 nm) than the repair-competent strain B/r, whilst the *uvrA*, *recA* double mutant, *E. coli* K12 AB2480, showed a marked increase in near-UV radiation sensitivity. Thirdly, inhibitors of repair of far-UV radiation-induced damage also sensitize wild-type cells to near-UV radiation. Peak (1970) showed that *E. coli* B/r was more sensitive to 365 nm radiation when plated in the presence of acriflavin (which interferes with both excision and recombination repair). This effect was also shown for *uvrA*, *recA* and repair-proficient strains by Webb and Brown (1976).

DNA Repair Systems

There is evidence that DNA repair systems are inactivated by high fluences of near-UV radiation. The capacity of the photoreactivating enzyme to monomerize pyrimidine dimers was inactivated by near-UV radiation at fluences that inactivate repair-proficient strains of *E. coli* (Tyrrell *et al.*, 1973). Both excision repair and recombination repair have been suggested as processes that are significantly impared by high 365 nm radiation fluences (Tyrrell and Webb, 1973; Webb *et al.*, 1978). Tyrrell (1974) demonstrated that near-UV radiation (365 nm) can inhibit the repair of DNA single-strand breaks induced by X-irradiation of *E. coli*. Tyrrell (1979) also estimated that with *E. coli* K12 (wild type) the shoulder on the near-UV radiation survival curve ended at approximately $10^{6} Jm^{-2}$. This fluence would have produced about 30 single-strand breaks in the DNA of a dark-repair deficient strain. These breaks are presumably repaired below this fluence in the wild type strain and the subsequent decrease in survival at higher fluences would indicate the destruction of repair systems.

RDP Reductase

Peters (1977) demonstrated that the enzymatic activity of the ribosyl diphosphate reductase (RDP) complex is inactivated in exponential repair-competent *E. coli* cells by broad-band, near-UV radiation at fluences comparable to these necessary for killing. In *E. coli* this enzyme complex is responsible for the *de novo* synthesis of deoxyribonucleotides. Deoxyribonucleotides can be synthesized by an alternative pathway if exogenous deoxyribose is provided. Peters (1977) demonstrated that the addition of deoxyadenosine or of deoxyadenosine plus thymine, but not of adenine alone, protected cells against lethality. However, it is not known what part of the RDP reductase complex is sensitive.

Chromophores for Near-UV Radiation Lethality

The chromophore for a biological effect is the molecule or molecular group whose absorption of radiation leads to the biological effect in question, in many cases cell inactivation. DNA does absorb into the near-UV radiation region (Sutherland and Griffin, 1981) and pure, transforming DNA is inactivated throughout this region (Peak *et al.*, 1973: Cabrera-Juárez *et al.*, 1976). However, for the reasons discussed above, the direct absorption of DNA is unlikely to be the major contributing lethal effect of near-UV radiation. Jagger (1985) suggests that under anaerobic conditions near-UV killing may be due to direct DNA absorption whereas aerobic killing is due to absorption by other molecules tightly bound to DNA that require oxygen to produce their lethal photoproducts. Compounds that quench excited molecules or that scavenge free radicals such as histidine, 2-aminoethylisothiouronium bromide hydrobromide (AET) and diazobicyclo (2.2.2.) octane (DABCO) provide protection against near-UV but not far-UV inactivation of transforming principle (Peak *et al.*, 1973: Peak and Peak,

1975; Tyrrell *et al.*, 1974: Peak *et al.*, 1981) supporting the idea that near-UV radiation lethal effects are not due to direct absorption by DNA.

The plateaux seen in the action spectrum for killing of stationary phase *E. coli.* in air (fig. 5) also suggest absorption by specific chromophores. These shoulder regions are not seen when cells are irradiated in the absence of oxygen, again suggesting different mechanisms for aerobic and anaerobic killing. Work by Tsai and Jagger (1981) suggests that the 340 nm plateau in the action spectrum for aerobic killing is due to the absorption by 4-thiouridine (^{4}Srd). This idea is supported by observations made by Peak *et al.* (1984) that showed the addition of ^{4}Srd to a solution of transforming DNA before irradiation at 334 nm, greatly increased the number of single-strand breaks seen. Absorption by protein B2 of the RDP reductase complex may account for the 410 nm plateau (Peters, 1977: Jagger, 1985).

Lethal Lesions for Near-UV Radiation Lethality

Pyrimidine Dimers

Pyrimidine dimers are induced in bacterial DNA by 365 nm radiation (Tyrrell, 1973). The ratio of dimer induction at 254 nm to that at 365 nm, based on energy fluence, was found to be 7.1×10^5, which is close to the ratio of 9×10^5 for inactivation of the double repair-deficient mutant *E. coli* K12 AB2480 (*uvrA, recA*) (Brown and Webb, 1972). However the ratios of thymine-thymine dimers to uracil-thymine dimers are different for 254 nm and 365 nm irradiation, with the former giving a $\widehat{TT}$ to $\widehat{UT}$ ratio of 1.1 and the latter a ratio of 5.0. Results obtained after 365 nm irradiation at 0°C show subsequent photoreactivation of cells at 25°C, but when cells are irradiated under conditions of low fluence rate at room temperature in air, photoreactivation is not seen. As 365 nm is within the efficient region of

the action spectrum for photoreactivation, cells irradiated with near-UV undergo concomitant PR and dimers are completely repaired during irradiation. For these reasons, pyrimidine dimers are not considered a major lethal lesion for near-UV radiation.

Single-Strand Breaks

Near-UV radiation also induces single-strand breaks (alkali-labile bonds) in bacterial DNA (Tyrrell *et al.*, 1974). Action spectra for *in vivo* induction of single-strand breakage in DNA have been obtained for *Bacillus subtilis* (Peak and Peak, 1982) and for human fibroblasts (Rosenstein and Ducore, 1983). There is circumstantial evidence to suggest that single-strand breaks may be the lesion of primary importance in near-UV inactivation. Firstly the induction of single-strand breaks at 365 nm is oxygen dependent (Tyrrell *et al.*, 1974: Webb, 1977) as are the induction of near-UV lethal events. Secondly, single-strand breaks occur at 405 nm. This wavelength induces lethality but does not produce dimers. Webb (1977) has estimated that the relative number of dimers per single-strand break is 800 at 254 nm, 1.8 at 365 nm and less than 0.09 at 405 nm. Thirdly, single-strand breaks are rapidly repaired after holding the irradiated cells in buffer at 30°C (Ley *et al.*, 1978). This capacity for repair is reduced in polymerase I deficient mutants. These mutants also show increased near-UV radiation sensitivity when compared to the *pol*$^+$ parent strain (Ley *et al.*, 1978: Yoakum, 1975) which suggests the involvement of dark-repair systems. Finally, the addition of ^{4}Srd to a solution of bacterial DNA in phosphate buffer greatly enhances single-strand breakage (Peak *et al.*, 1984). Since the presence of ^{4}Srd has been shown to play an important role in near-UV lethality in *E. coli* B/r (Tsai and Jagger, 1981) it is likely that ^{4}Srd is responsible for a considerable fraction of the single-strand breakage produced in bacterial DNA at 334 nm. It is also

probable that absorption by ^{4}Srd leads to photosensitized induction of single-strand breaks in DNA, which is a different mechanism than that responsible for ^{4}Srd induced growth delay (Jagger, 1985: and see near-UV sublethal effects below).

Other DNA Lesions

Lesions other than pyrimidine dimers and single-strand breaks may also contribute to near-UV radiation induced cell lethality. Cabrera-Juárez and Setlow (1977) found an adduct that was similar to, but not identical to, the spore photoproduct (5-thyminyl - 5, 6-dihydrothymine) after near-UV irradiation of DNA. The pyrimidine adduct is unlikely to contribute to near-UV lethal damage in DNA as it is only produced at low efficiency. Cross-linking between DNA and protein has been induced by visible light in the presence of acridine orange (Smith, 1976) and after the combined near-UV radiation and H_2O_2 inactivation of phage T7 (Hartman *et al.*, 1979). DNA-protein crosslinking has also been reported in normal human skin fibroblasts exposed to near-UV (solar) radiation (Lai *et al.*, 1987). However, these and other DNA lesions appear to have only a minor role in near-UV lethality.

In Membranes

Some near-UV lethality may result from membrane alterations. Moss and Smith (1981) showed that stationary phase *E. coli* K12 irradiated with broad-band near-UV exhibited lower survival if plated on minimal rather than complex medium. This effect was oxygen dependent and appeared to reflect membrane damage since it was also dependent on the NaCl concentration in the minimal medium. Kelland *et al.* (1983 a and b) confirmed this finding and reported that such cells would recover if held

in complex recovery medium. Recovery was inhibited by bacitracin but not chloramphenicol or penicillin implying that membrane synthesis but not protein or cell wall synthesis was required for recovery. Further work by Kelland *et al.* (1984a) showed that cells exhibited leakage of ^{3}H-thymidine and $^{86}Rb^{+}$ after near-UV but not far-UV irradiation and that there was good correlation between leakage and cell lethality.

Klamen and Tuveson (1982) suggested that membrane damage and the resultant lethality arose from near-UV generated singlet oxygen reacting with the unsaturated phospholipids. They tested this proposal in *E. coli* K1060, a strain unable to synthesize or degrade unsaturated fatty acids, by growing the cells with fatty acid so that the fatty acid would be incorporated into the membrane. They found that the fatty acid composition of the membrane influenced near-UV radiation sensitivity in exponential phase cells. Cells were more sensitive when they contained fatty acid with a higher degree of unsaturation. This could be the result of singlet oxygen attack on double bonds leading to increased lipid peroxidation. This was confirmed by Chamberlain and Moss (1987) who showed, also using K1060, that membrane damage (measured by $^{86}Rb^{+}$ leakage) increased in cells with fatty acid with a high degree of unsaturation. This was paralleled by an increased production of lipid peroxidation products. Both lipid peroxidation and $^{86}Rb^{+}$ leakage increased in D_2O (which prolongs the lifetime of singlet oxygen) which would again implicate singlet oxygen. However, the opposite effect was reported by Wagner *et al.* (1980) for K1060 exposed to near-UV radiation in the presence of the photosensitizer acridine. They suggested that a high degree of unsaturation in lipid would lead to increased membrane fluidity which would be less susceptible to disruption by near-UV radiation.

Further recent evidence for the involvement of the membrane in near-UV radiation inactivation has been provided by Kralli and Moss (1987). They showed the addition of Trolox-C, a vitamin E analogue, to the pre-irradiation growth medium or the post-irradiation plating medium protected a near-UV sensitive strain of human fibroblasts against 365 nm irradiation. Vitamin E can act as a general antioxidant and has a stabilizing effect on cell membranes. It can also scavenge species such as singlet oxygen and is effective in stopping lipid peroxidation. This suggests that Trolox-C prevents membrane damage by stopping lipid peroxidation of the membrane.

From these results, it is clear that near-UV radiation damages cell membranes. However, the extent of the effect of this damage on survival is dependent on the growth phase of the cells and the post-irradiation incubation conditions.

Growth Phase Effects

Sensitivity to the effects of near-UV radiation is greatly dependent on the growth phase of cells. Exponential phase cells are often much more sensitive than stationary phase cells to near-UV inactivation. Harrison (1967) and Peak (1970, represented in fig. 6A) reported that cells of repair-proficient strains of *E. coli* were more sensitive to near-UV radiation in exponential phase than in stationary phase. This result has also been shown for other repair-proficient strains of *E. coli* (Tuveson and Jonas, 1979, for AB1157: Hartman and Eisenstark, 1978, for W3110) and in other cells such as *Trichomonas vaginalis* (Daly *et al.*, 1981) and mouse myeloma (Griego *et al.*, 1981, represented in fig. 6B). This growth phase effect has also been shown with *recA, relA* and *recA, nur* strains of *E. coli*

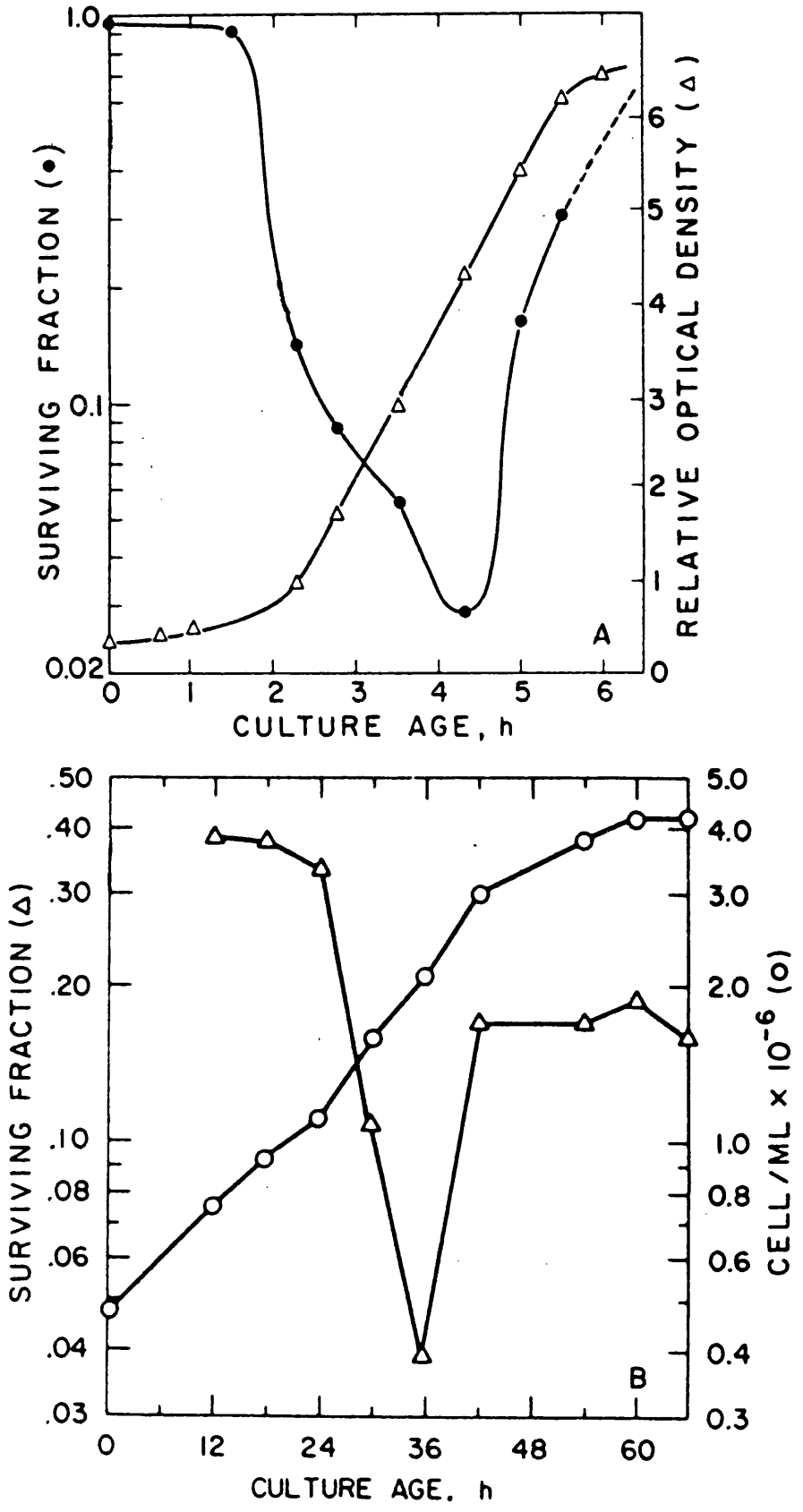


Figure 6: Sensitivity of (A) *E. coli* B/r and (B) mouse myeloma cells to 365 nm monochromatic near UV radiation as a function of growth phase. (Adapted from (A) Peak, 1970 and (B) Griego *et al.*, 1981.)

(Tuveson and Jonas, 1979) and in *recA* strains of *E. coli* and *S. typhimurium* (Eisenstark,1970, 1973: Mackay *et al.*, 1976).

However, greater sensitivity of exponential phase cells is not always seen. Webb and Peak (cited by Webb, 1977) tested the near-UV radiation (365 nm) sensitivity of four *E. coli* strains at different stages of growth and found that the repair competent and *recA* strains were more sensitive in exponential phase, whereas *uvrA* and *uvrA,recA* strains showed approximately equal sensitivity in both stationary and exponential phases of growth. This was also observed with excisionless strains of *E. coli* after 254 nm irradiation (Tyrrell *et al.*, 1972) where a strain deficient in excision repair but proficient in recombination repair showed decreased sensitivity during exponential growth. Kelland *et al.* (1984b) have also reported that some repair-competent *E. coli* strains are more sensitive to near-UV radiation in stationary phase than in exponential phase.

Factors other then DNA repair are also affected by phase of growth. Moss and Smith (1981) and Kelland *et al.* (1983a, b, 1984a) found salt dependent near-UV radiation induced membrane damage in stationary phase cells, whilst Ito and Ito (1983) found similar correlation in yeast but mainly for cells in exponential phase. Klamen and Tuveson (1982) also found that the effects of unsaturated fatty acids on sensitivity to near-UV radiation was only important in exponential phase cells. Furthermore, the differences in sensitivity of exponential and stationary phase cells are greater for inactivation by broad-band near-UV radiation than for monochromatic radiation (Eisenstark, 1970: Mackay *et al.*, 1976).

The differences seen between stationary and exponential phase cells in terms of near-UV radiation sensitivity suggest that different mechanisms of inactivation and repair are involved at different stages of cell growth. Mechanisms important for stationary phase cells may become relatively

unimportant in exponential phase cells and *vice versa*. Thus it is important to use cells at a very specific phase of growth when studying the effects of near-UV radiation and to extend studies to include different defined phases of growth.

Indirect Effects of Near-UV Radiation

Some near-UV radiation lethality may involve DNA damage by activated atoms or molecules in the intracellular medium. These are likely to be active oxygen species, such as superoxide radicals, singlet oxygen and hydroxyl radicals, since near-UV radiation inactivation is largely oxygen dependent and scavengers of oxygen species, such as histidine, AET and DABCO, provide protection against near-UV radiation (see section on chromophores for near-UV radiation lethality). The role of reactive oxygen species, oxidative stress and its inducible repair will be discussed at greater length in the second part of the introduction.

Repair of Near-UV Radiation Damage

Bacteria that are completely deficient in dark-repair systems for far-UV radiation damage are also sensitive to near-UV radiation. Webb *et al.* (1976) showed that strains carrying *uvrA*, *recA* and *polA* were more sensitive to near-UV radiation than repair-competent strains such as K12 AB1157 and that the double repair mutant K12 AB2480 (*uvrA*, *recA*) showed even greater sensitivity to near-UV radiation. Although the range of sensitivities of these mutants to 254 nm radiation was greater than that shown at 365 nm (Webb, 1977), the differences show that repair capability is an important factor in the lethal effects of near-UV radiation. However, some workers have reported that strains lacking either only recombination repair or only excision repair are as resistant to near-UV radiation as repair competent

strains (Tuveson and Jonas, 1979: Tuveson, 1980) suggesting that either system alone can be very efficient in repairing near-UV radiation damage.

Thus, repair systems that operate for far-UV radiation damage also operate in near-UV radiation inactivation. However, cells have other mechanisms for defence against near-UV radiation in addition to the far-UV repair systems. It is not surprising that additional mechanisms are required in view of the range of targets, chromophores and lesions involved in near-UV radiation damage. These additional mechanisms will be discussed in the second part of the Introduction.

Near-UV Radiation Sublethal Effects

Owing to the relative inefficiency of near-UV radiation as compared to far-UV, effects which are overshadowed by lethality in the far-UV region become more apparent with near-UV radiation. Many of these effects occur at sublethal fluences, that is at fluences approximately one order of magnitude below those required for lethality.

The first sublethal effect to be recognised was that of growth inhibition. This consists of (a) a growth delay (i.e. a delay in the onset of growth) lasting approximately 90 min. and (b) a growth rate depression (i.e. a slight decrease in the rate of growth once growth resumes). Growth inhibition can be induced by low fluences of near-UV radiation or sunlight (Jagger, 1975). Hollaender first reported near-UV radiation induced inhibition of bacterial growth in nutrient broth in 1943. The effect was also shown to occur on nutrient agar plates (Jagger *et al.*, 1964).

Action spectra for near-UV induced growth delay show a narrow effective range of wavelengths from 300 to 380 nm with a peak effect around 340 nm (Jagger *et al.*, 1964) indicating the involvement of a specific

chromophore other than DNA. Work by Ramabhadran and Jagger (1975) extended this idea and showed that near-UV induced growth inhibition in *E. coli* K12 strains was not photoreactivable and was not affected by far-UV dark repair systems. This also suggested that DNA damage was not involved.

Favre *et al.* (1975) showed that 334 nm irradiation of *E. coli* $tRNA^{VAL}$ resulted in the formation of a cross-linked adduct between 4-thiouridine (^{4}Srd), an unusual nucleoside occuring at the 8-position in about 65% of *E. coli* tRNAs (Carré *et al.*, 1974), and the cytidine residue at position 13 (see diagram 1 below). The absorption spectra of ^{4}Srd when present in

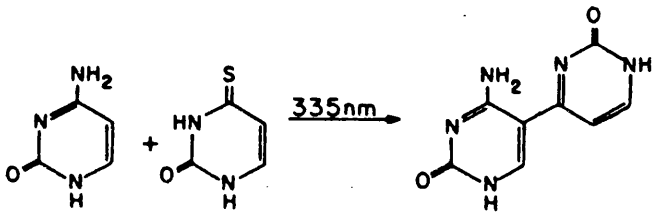


Diagram 1: Formation of 4-thiouracil-cytosine adduct

$tRNA^{VAL}$ (Favre *et al.*, 1971) fits closely with the action spectra seen for near-UV induced growth delay (Ramabhadran and Jagger, 1975; and see fig. 7). Ramabhadran (1975) concluded that ^{4}Srd was the major chromophore for growth delay in *E. coli* and that the ^{4}Srd-Cyd adduct was the probably photoproduct. This was confirmed by Thomas and Favre (1975) for growth delay in *E. coli* B/r.

Ramabhadran (1975) also noted that near-UV irradiation of cells halted RNA synthesis and that the action spectra for RNA synthesis was similar to

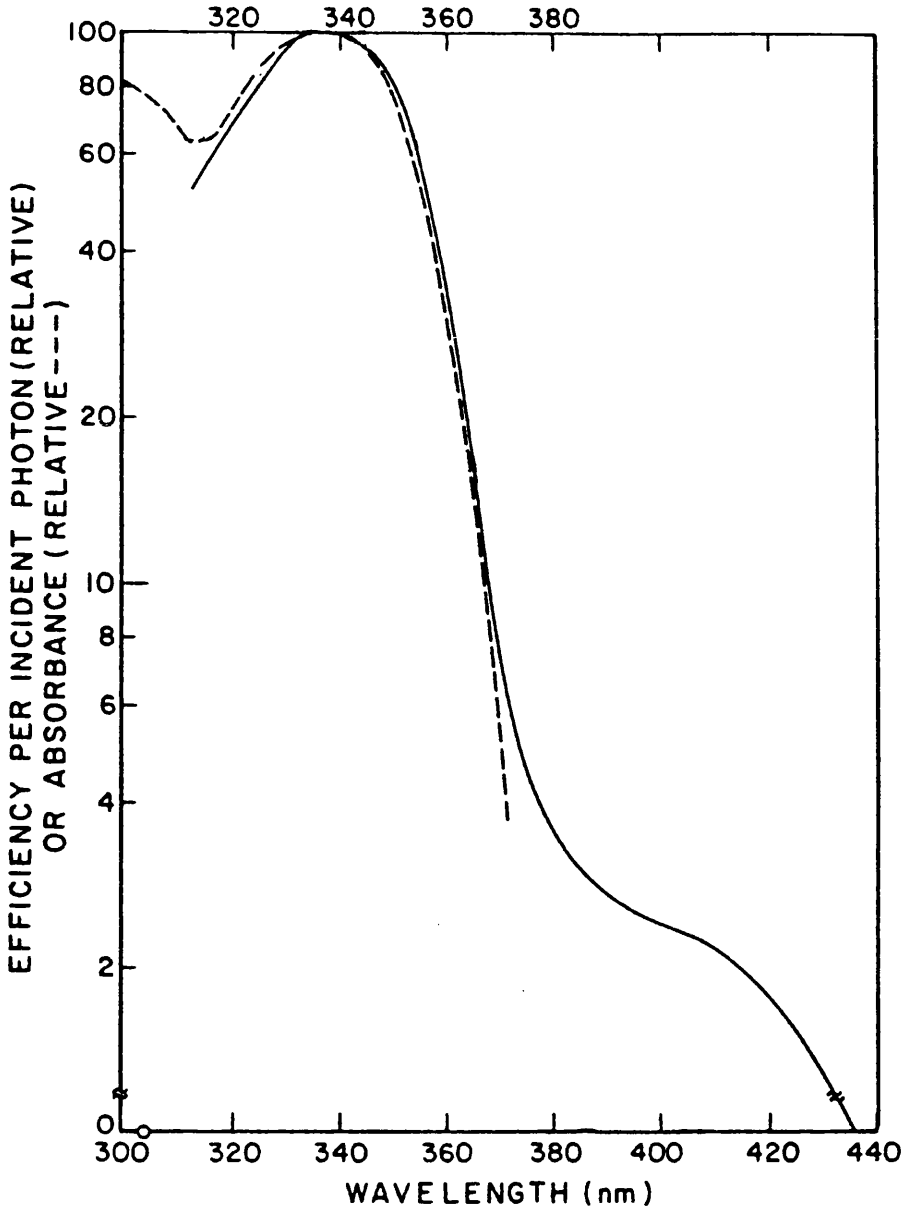


Figure 7: Action spectrum for growth inhibition in *E. coli* B (solid line) and absorption spectrum for *E. coli* $tRNA^{VAL}$ (broken line). (Adapted from Jagger, 1985.)

that of growth delay. He proposed that growth delay may involve the stringent control of RNA synthesis.

Stringent control is the collection of adjustments made by *E. coli* cells when faced with an imbalance in growth conditions, such as amino acid starvation (for a review see Gallant, 1979). When *E. coli* is starved of a required amino acid, the uncharged tRNAs produced cause the ribosome to produce ppGpp (guanosine tetraphosphate). This causes a block in transcription of stable RNA (tRNA and rRNA) resulting in the cessation of stable RNA synthesis. Stringent control is not seen in relaxed (rel^-) strains (Cashel, 1969).

Ramabhadran and Jagger (1976) observed that rel^- strains showed no growth delay after near-UV irradiation. Furthermore they showed that ppGpp was produced after near-UV irradiation of rel^+ strains but not of rel^- strains. Thus it appeared that near-UV radiation simulated amino acid starvation. Absorption by ^{4}Srd and the subsequent formation of the ^{4}Srd-Cyd adduct in a small fraction of the total RNA induced the stringent response resulting in complete cessation of RNA and protein synthesis. This in turn would lead to an extended growth delay. The role of ^{4}Srd was further confirmed by Tsai and Jagger (1981) who showed that mutant strains lacking ^{4}Srd showed no growth inhibition after broad-band near-UV irradiation.

Other sublethal effects have also been described; the reduction of capacity to support phage growth and the inhibition of induced tryptophanase synthesis; these also involve ^{4}Srd. Most of the work on growth delay has been carried out with *E. coli* B/r and B strains. There is evidence that *E. coli* K12 strains may respond differently as a secondary

chromophore for growth delay, ^{2}Srd, has been identified (Thomas *et al.*, 1981).

Near-UV sublethal actions have been reviewed by Jagger (1985) and Favre *et al.* (1985).

PART II

The Role of Oxidative Stress in Near-UV Radiation Induced Damage

In the first part of the Introduction, the main effects of near-UV radiation on living organisms have been discussed; these covered the targets, chromophores and lesions involved in near-UV radiation lethality. The overall picture which emerges is that DNA appears to be a major target for near-UV radiation killing of cells under most circumstances, with single-strand breaks being a likely lesion. Endogenous photosensitizers such as ^{4}Srd seem to play an important role. Marked differences between near-UV and far-UV killing are seen; most noticeably that the low efficiency of near-UV killing, as compared to far-UV killing, leads to a variety of mechanisms being involved in inactivation and repair. Secondly, near-UV radiation, but not far-UV, has a high-oxygen demand for killing. This oxygen dependence suggests that lethal effects at wavelengths longer than 320 nm may involve photodynamic (photo-oxidative) processes mediated by endogenous sensitizers which ultimately lead to DNA damage.

This damage to DNA is subject to a number of enzymatic repair systems some of which, in particular that controlled by the *recA* gene, are inducible, that is, the enzymes are only produced in effective concentrations in response to the formation of damage. Inducible repair is also produced after stresses other than near-UV radiation such as following heat shock, or the SOS response following far-UV irradiation and there is evidence that there is considerable overlap between these difference stresses (Morgan *et al.*, 1987: Van Bogelen *et al.*, 1987).

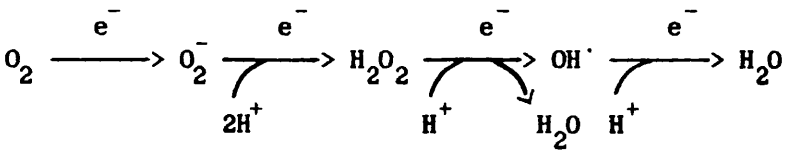
The remainder of the introduction examines the role of oxidative stress and its inducible repair in near-UV radiation lethality.

The Biological Effects of Reactive Oxygen Species

In order to understand the mechanisms of oxidative damage and stress on cells, it is first useful to make some general comments about oxygen toxicity and the free radicals that are generated in oxygen mediated reactions.

The Generation of Reactive Species

Oxygen is essential to all respiring organisms, yet it is also toxic, causing oxidations within the cell that may be harmful or even lethal. Ground state oxygen contains two unpaired electrons which, by definition, means that oxygen itself is a free radical, that is, a species capable of independent existence that contains one or more unpaired electrons. However, these electrons are in parallel spin, which means that oxygen cannot accept paired electrons from another atom or molecule. This spin restriction means that the reduction of oxygen favours univalent pathways. It also makes oxygen relatively unreactive and most photodynamic effects are thought to occur via the reactive oxygen intermediate species formed during the reduction of oxygen to water, as shown in the equation below:



The single electron addition to molecular oxygen results in the production of the superoxide radical (O_2^-). The addition of a further electron results in the peroxide ion (O_2^{2-}). In biological systems this is usually protonated to give hydrogen peroxide (H_2O_2). Hydrogen peroxide decomposes easily to give the hydroxyl radical ($OH^{\cdot}$) and this may then be reduced to water (H_2O).

A further reactive oxygen species is singlet oxygen ($^1O_2$), in which the spin restriction of ground state oxygen has been removed by pairing the two normally unpaired electrons. Singlet oxygen is therefore not a free radical, but the removal of the spin restriction increases its oxidizing ability making it more reactive than ground state oxygen.

Toxicity of Reactive Species

As stated above, the removal of the spin restriction in singlet oxygen makes it a reactive species. It is important in photobiology as it can be easily generated in photosensitization reactions. These occur when UV absorbent compounds, such as acridine orange and rose bengal, are irradiated with light of a given wavelength. The radient energy is absorbed and the energy raises the sensitizer molecules into an excited state. The excitation energy is then transferred to an adjacent oxygen molecule converting it to singlet oxygen and the photosensitizer returns to its ground state. The singlet oxygen produced can then react with other molecules and the changes produced are known as photodynamic effects. Many compounds formed *in vivo* are effective sensitizers such as the vitamin riboflavin, the bile pigment bilirubin and various porphyrins. Singlet oxygen also reacts chemically with many biological compounds including DNA, proteins and lipids, resulting in multiple reaction products.

The superoxide radical also damages a wide range of biological materials including DNA. However, in aqueous solutions it is only a weak oxidizing agent as it undergoes dismutation:

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

Haber and Weiss (1934) suggested that the toxicity of O_2^- was due to O_2^- and H_2O_2 combining in the presence of a metal catalyst to produce the reactive

hydroxyl radical, $OH^{\cdot}$:

$$H_2O_2 + O_2^- \xrightarrow[\text{catalyst}]{\text{metal}} O_2 + OH^- + OH^{\cdot}$$

Once generated, the hydroxyl radicals react with molecules in their immediate surroundings. The less reactive O_2^- and H_2O_2 can, however, diffuse away from their sites of formation and generate $OH^{\cdot}$ in different parts of the cell whenever they meet a metal ion catalyst.

Any system generating O_2^- will produce H_2O_2 by the dismutation reaction. Hydrogen peroxide is also a normal metabolite of cells. However, although H_2O_2 is only a weak oxidizing agent, it is also toxic. This toxicity is though to arise when H_2O_2 is reduced intracellularly in the presence of iron (II) or copper (I) salts, in what is known as a Fenton reaction, to produce a series of radicals; for example:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

$$OH^{\cdot} + H_2O_2 \longrightarrow H_2O + H^+ + O_2^-$$

$$OH^{\cdot} + Fe^{2+} \longrightarrow Fe^{3+} + HO^-$$

The main reactivity is likely to be due to the hydroxyl radical, $OH^{\cdot}$.

Hydrogen peroxide is produced during near-UV irradiation of bacteria and viruses and, as will be discussed later, the toxic effects seen appear to be due to DNA damage, especially single-strand breaks.

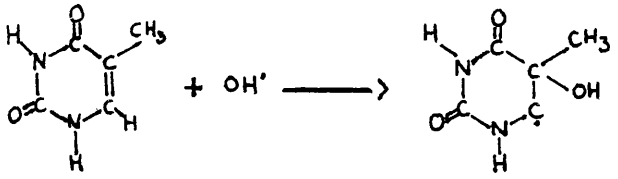
The hydroxyl radical, $OH^{\cdot}$, is extremely reactive and reacts with almost every type of molecule of biological importance. It reacts by hydrogen abstraction, addition and electron transfer. For example:

(a) hydrogen abstraction:

$CH_3OH + OH^{\cdot} \longrightarrow {}^{\cdot}CH_2OH + H_2O$

methanol

(b) addition:



thymine

(c) electron transfer:

$Cl^- + OH^{\cdot} \longrightarrow Cl^{\cdot} + OH^-$

chloride ion

Further radicals are formed by the reactions of $OH^{\cdot}$, but these tend to be less reactive than $OH^{\cdot}$.

Protection against Free Radicals

Most cells contain a wide variety of enzymes that protect them against damage by oxygen radicals. In respiring, wild-type bacteria this is carried out by three main classes of enzyme. Firstly there are the catalases which catalyse the decomposition of H_2O_2 to water. Secondly, there are peroxidases which catalyse the decomposition of H_2O_2 to water in the presence of suitable substrates. These two classes of enzyme control the amount of H_2O_2 that is allowed to accumulate within the cell. Thirdly, there is the enzyme superoxide dismutase. This was first identified by McCord and Fridovich (1968) and is found in virtually all cells. It acts

specifically in the dismutation of the superoxide radical to H_2O_2 and oxygen.

Other mechanisms are also employed, especially in higher organisms, such as the conjugated diene system found in melanin and carotenoids that helps to quench reactive species.

The role of free radicals in biology has been extensively reviewed in 1976 in a series of volumes edited by W. Pryor and more recently in a book by Halliwell and Gutteridge (1985).

The Role of Hydrogen Peroxide in Near-UV Radiation Effects

In 1972 Yoakum and Eisenstark showed that the near-UV irradiation of tryptophan yielded a photoproduct that killed recombination deficient (*rec*) mutants of *Salmonella typhimurium*. The production of this lethal photoproduct was oxygen dependent. Yoakum and co-workers also found that this photoproduct inhibited the repair of DNA single-stand breaks and DNA replication gap closure in *E. coli* (Yoakum *et al.*, 1974) and that it sensitized bacteria to near-UV radiation-induced DNA strand-breaks (Yoakum, 1975). This lethal photoproduct of tryptophan was identified as hydrogen peroxide (McCormick *et al.*, 1976). McCormick *et al.* (1976) also showed that H_2O_2 caused the same biological effects as near-UV radiation. This was confirmed by Anatheswamy and Eisenstark (1976) who showed that both the typtophan photoproduct and H_2O_2 exerted identical biological actions on T7 phage. Furthermore, a 50- to 100-fold synergistic effect was found between near-UV radiation and H_2O_2 in the killing of both *E. coli* (Hartman and Eisenstark, 1978) and T7 phage with enhancement showing a peak at 340 nm (Anatheswamy *et al.*, 1979). Synergistic mutagenesis was also reported between broad-band near-UV radiation and pre-irradiated tryptophan in *E. coli* (Hartman and Eisenstark, 1978).

A sequence of events that could lead to near-UV radiation induced production of H_2O_2 and subsequent lethality has been proposed (McCormick and Oczós, 1979: Walrant and Santus, 1974). This suggests that

(a) 310 nm light is absorbed by tryptophan.

(b) In the presence of oxygen, the tryptophan is converted to N-formylkynurenine which absorbs at 317 nm, gaining an excited state and promoting the photo-oxidation of DNA.

(c) N-formylkynurenine also promotes the formation of singlet oxygen and superoxide anion leading to the formation of H_2O_2.

(d) The H_2O_2 produced enhances the damage to DNA.

Although this sequence of events is still partly speculative, it does suggests how tryptophan and H_2O_2 are involved in near-UV radiation effects. The H_2O_2 produced enhances already damaged DNA and H_2O_2 itself has been shown to produce transient radical species which cause oxidative DNA damage (Demple and Linn, 1982).

Endogenous Protection against Near-UV Radiation Induced Oxidative Stress

The Role of Catalase

If, as has been suggested, H_2O_2 were an important product of near-UV radiation in bacteria, it would be expected that cells lacking the ability to cope with H_2O_2 would be sensitive to inactivation by near-UV radiation. One of the major mechanisms for protection of cells against the harmful effects of H_2O_2 is the enzymatic decomposition of H_2O_2 by catalase.

Bacterial Catalases

Catalase (E.C.1.11.1.6) is present in almost all aerobically respiring prokaryotic and eukaryotic cells and was one of the first enzymes to be isolated to a high state of purity. Catalase is the enzyme catalysing the decomposition of H_2O_2:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

In the presence of suitable substrates, catalase may also act as a peroxidase:

$$RH_2 + H_2O_2 \xrightarrow{\text{peroxidase}} R + 2H_2O$$

Most catalases are tetrameric hemoproteins of high efficiency with high catalytic and low peroxidatic activity. Reviews of the structure, function and biosynthesis of catalase are available (for examples see Schonbaum and Chance, 1976; Ruis, 1979; Fita and Rossmann, 1985).

In *E. coli* two electrophoretically distinct catalases have been identified and labelled HPI and HPII (Claiborne and Fridovich, 1979). Genetic mapping studies have confirmed the presence of the two independent species of catalase in *E. coli*. The locus *katG* affects the synthesis of the bifunctional isoenzyme pair HPI-A and HPI-B (Loewen *et al.*, 1985a) that possess both catalase and perioxidase activities (Claiborne and Fridovich, 1979). The loci *katE* (Loewen, 1984) and *katF* (Loewen and Triggs, 1984) affect the synthesis of the monofunctional catalase HPII (Loewen and Triggs, 1984).

Induction of Catalase by H_2O_2

Catalase has been shown to be induced by H_2O_2. Finn and Condon (1975) demonstrated that addition of H_2O_2 to *Salmonella typhimurium* cultures, grown in defined medium, within the range 1 µM to 2 mM during exponential growth, stimulated catalase synthesis. The extent of catalase synthesis was found to be dependent on the concentration of H_2O_2, with a maximum response at 80 µM. The effect was not detected, however, until 10 to 15 minutes after the addition of H_2O_2. A similar effect was reported in *E. coli* B23 by Richter and Loewen (1981), with both H_2O_2 (20-120 µM) and ascorbate (0.57-5.7 mM) increasing catalase activity. Demple and Halbrook (1983) also found that 30 µM H_2O_2 added to exponentially growing *E. coli* K12 AB1157 caused an increase in catalase activity; however, the increase in activity was variable.

In general, catalase activity is a poor indicator of sensitivity to H_2O_2. Catalase activity is variable throughout growth and has been shown to decrease during exponential growth and increase at the onset of and during stationary phase for both *S. typhimurium* and *E. coli* (Finn and Condon, 1975). Yosphe-Purer and Henis (1976) showed that culture medium, growth phase and temperature play important roles in the sensitivity of *E. coli* to H_2O_2 by affecting the levels of catalase synthesised. A correlation of catalase level and H_2O_2 sensitivity could only be seen when the H_2O_2 concentration was not excessive in proportion to the amount of catalase available. Catalase has been reported to exhibit a linear relationship between reaction rate and substrate concentration upto high H_2O_2 concentrations (50 mM) at which point the enzyme starts to inactivate (Nies and Schlegel, 1984). Some workers have used high H_2O_2 concentrations (50 mM) with no apparent problems (e.g. Sammartano *et al.*, 1986).

Catalase appears to be regulated by catabolite repression (Hasson and Fridovich, 1978; Yosphe-Purer *et al.*, 1977) as glucose suppresses the synthesis of catalase but the effect is reversed by the addition of cyclic-AMP to the growth media (Hasson and Fridovich, 1978). As a result of this, exhaustion of the glucose present in a complex medium or abrupt transfer of cells from a glucose-containing to a glucose-free medium results in a sharp increase of catalase activity (Hasson and Fridovich, 1978). The variability of catalase activity resulting from different experimental methods may account for the differences in correlation between H_2O_2 sensitivity and catalase levels reported by various workers.

Another reason for seemingly anomalous results may arise from different behaviours of the two bacterial catalases, HPI and HPII. The bifunctional catalase, HPI, has been shown to be induced by H_2O_2 whereas HPII was not; conversely levels of HPII increased during growth into stationary phase on some TCA-cycle intermediates whilst levels of HPI did not increase under the same circumstances (Loewen *et al.*, 1985b). Furthermore, HPI was shown to be controlled by the global regulatory locus *oxyR* that is involved in defense against oxidative stress (Christman *et al.*, 1985) whilst HPII does not respond to *oxyR* (Loewen *et al.*, 1985b).

Sensitivity of Catalase Deficient Mutants to Near-UV Radiation and H_2O_2

Demple and Halbrook (1983) used the addition of 30 µM H_2O_2 as a pretreatment to challenging *E. coli* with 5 mM H_2O_2. Although resistance was induced to 5 mM H_2O_2, the degree of resistance did not correlate with the increased catalase activity seen. This was similar to an earlier report by Carlsson and Carpenter (1980) who found no correlation between H_2O_2 toxicity (200 µM) in repair-deficient strains of *E. coli* K12 with either the capacity of intact cells to decompose H_2O_2 or with the level of

catalase in cell-free extracts. However, correlation between H_2O_2 toxicity and catalase activity has been reported (Winquist *et al.*, 1984).

Hydrogen peroxide pretreatment has also been shown to protect *E. coli* against subsequent near-UV radiation damage (Tyrrell, 1985; Sammartano and Tuveson, 1985). This would be expected if H_2O_2 is produced during irradiation and is causing the lethal effects of near-UV radiation. Tyrrell (1985) has also show that sublethal fluences of near-UV radiation induce protection against H_2O_2 inactivation, suggesting a common protective pathway. Hartman (1986) and Eisenstark *et al.* (1986) have also presented evidence that H_2O_2 is important in near-UV inactivation of *E. coli* and phage.

These reports would, then, indicate that bacterial mutants deficient in or unable to induce catalase should be sensitive to near-UV radiation as well as H_2O_2. This has been shown to be true in some cases. *Escherichia coli* strains carrying the *nur* gene have been shown to be sensitive to near-UV but not far-UV radiation (Tuveson and Jonas, 1979; Tuveson, 1980; Tuveson, 1981). Recently the same group of workers have shown that the *nur* gene is identical to *katF* (Sammartano *et al.*, 1986) and that mutants carrying *katF* but not *katE* or *katG* are sensitive to broad-band near-UV radiation as well as H_2O_2. This is supported by preliminary data given by Tyrrell (1985) for the *E. coli katE* mutant UMI, which was not sensitive to either H_2O_2 or near-UV radiation treatments carried out in buffer. Eisenstark and Perrot (1987, and see fig. 9, towards the end of the introduction) have also found that *E. coli katF* mutants are near-UV radiation sensitive but *E. coli katG* and *katE* mutants show only slight near-UV radiation sensitivity. Likewise an *oxyR* mutant of *S. typhimurium* which constitutively synthesises increased catalase levels (Christman *et*

al., 1985) shows resistance to H_2O_2 but comparatively less resistance to near-UV radiation (Eisenstark and Perrot, 1987 and fig. 9).

These results indicate that whilst there are some similarities between the protective responses to near-UV radiation and H_2O_2 challenge, near-UV damage and recovery involves mechanisms in addition to those involving H_2O_2 alone.

The Role of Exogenous Catalase in Near-UV Radiation Protection

Although endogenous catalase may only have a minor role in protection against near-UV radiation damage in bacteria, very marked effects are seen when catalase is added exogenously. Sammartano and Tuveson (1984) incorporated catalase into the post-irradiation plating medium and found that this protected *E. coli* against the lethal and mutagenic effects of broad-spectrum near-UV radiation. Their results for near-UV inactivation are shown in fig. 8. Catalase protected against inactivation in all four strains tested. The presence of an alternative protein, amylase, in the plating medium did not protect against inactivation, indicating that the protection was not due to the presence of a protein but was due to the enzyme activity specific to catalase. Catalase added to the cell suspension during or immediately after near-UV radiation exposure also afforded protection against inactivation.

The same effect was shown in a very different system by Sah and Kesavan (1987). They showed that catalase incorporated in the growth medium after gamma irradiation afforded a concentration-dependent radioprotection in barley seeds incubated aerobically at 25°C. The effect was decreased at 4°C, when catalase activity is decreased, and eliminated by sodium azide, a catalase inhibitor. Exogenous catalase also plays a protective role in heat-stress. Addition of catalase to the plating medium

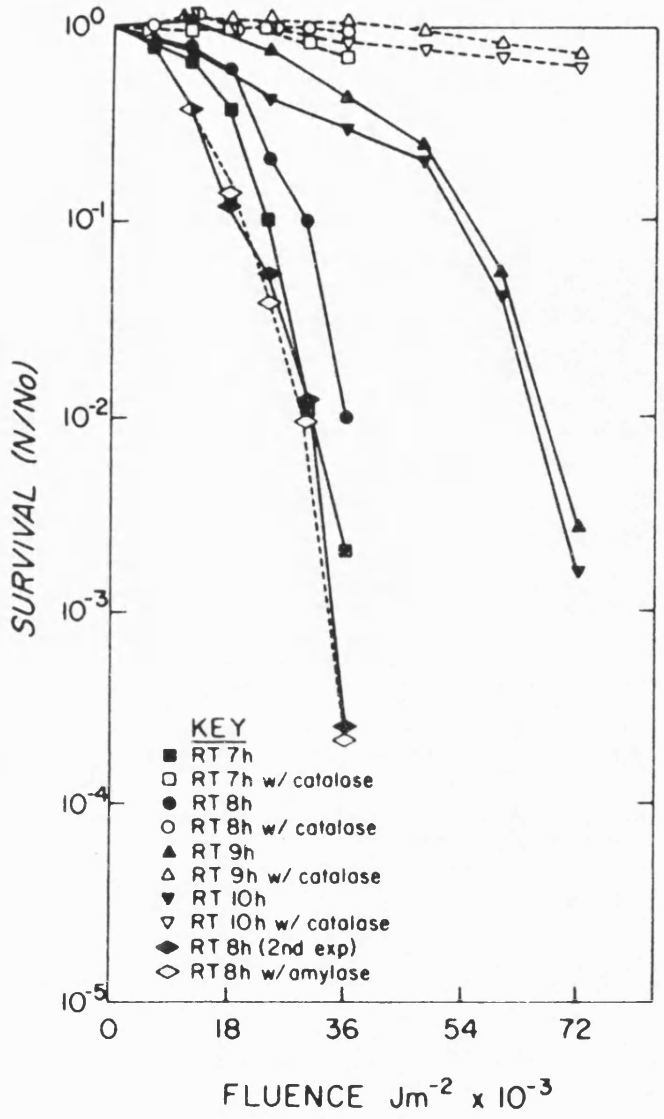


Figure 8: Survival curves for *E. coli* cells after near-UV irradiation. Cells were plated to media with catalase (10 µg/ml) or amylase (10 µg/ml) (dashed lines) or without any supplements (solid lines). (Adapted from Sammartano and Tuveson, 1984.)

of heat-injured *E. coli* DNA repair mutants increased survival to the level exhibited by the wild type strains (Mackey and Seymour, 1987) in agreement with earlier reports (Rayman *et al.*, 1978; Mackey and Derrick, 1982).

These examples of protection by exogenous catalase would suggest that catalase does have a role in oxidative damage in general and near-UV radiation damage in particular. This may reflect a central role for H_2O_2 in oxidative damage as other exogenous agents that decompose H_2O_2, such as pyruvate, also have a protective effect (Mackey and Derrick, 1982; Rayman *et al.*, 1978).

It would appear that catalase, both endogenous and exogenous, does have a role in protection against near-UV radiation-induced damage. However, this protection is complex and may differ from that seen for H_2O_2 challenge. Exogenous catalase may be able to prevent damage by decomposing H_2O_2 to water and oxygen thus preventing it being broken down to reactive oxygen species. Endogenous catalase may only have an important role under certain conditions. Induction experiments may induce catalase but also many other proteins (Christman *et al.*, 1985) which help in protection and repair.

The Role of DNA-Repair Function

Further evidence that catalase plays only a part in the protection of bacteria against near-UV radiation damage arises from the knowledge that certain DNA-repair mutants of "normal" catalase activity (e.g. *recA*, *xthA* and *polA*) are nonetheless sensitive to H_2O_2 and near-UV radiation.

The Role of *recA*

Bacterial mutants that are unable to undergo genetic recombination (*rec*$^-$) have been shown to be sensitive to H_2O_2 and both broad-band and monochromatic near-UV radiation.

In 1980, Carlsson and Carpenter reported that when a series of DNA-repair deficient *E. coli* strains were exposed to 200 µM H_2O_2, *recA* strains were killed more rapidly than both the repair-proficient and other repair-deficient strains (*recB, uvrA, B, C, recC, recF*). They also demonstrated that a functional RecA gene product was more important than the ability of the cells to enzymatically decompose H_2O_2 in protecting *E. coli* against the toxic effects of H_2O_2. Anatheswamy and Eisenstark (1977) also found an *E. coli recA* strain to be sensitive to H_2O_2 (10 mM). However, they showed that a *recA, recB* double mutant and a *polA* mutant were more sensitive to H_2O_2 than the single *recA* mutant. They concluded that the DNA lesion caused by H_2O_2 required both the *polA*$^+$ and the *recA*$^+$ pathways for repair. However, recent work by Sammartano *et al* (1986) showed inactivation by 50 mM H_2O_2 to be independent of the *recA* gene in *E. coli nur*$^+$ and *nur*$^-$ strains. Protection against H_2O_2 oxidative damage relied instead on the Nur and KatF gene product, catalase HPII (Sammartano *et al.*, 1986).

Earlier work by Tuveson and Jonas (1979) had shown that the *nur* strains used by Sammartano *et al.* (1986) were sensitive to broad-band and monochromatic near-UV radiation and that this sensitivity was independent of the *recA* gene. This does not agree with other workers who have found near-UV radiation sensitivity to be greater in *recA* strains than in their repair-competent, parent strains (Eisenstark and Perrot, 1987; Mackay *et al.*, 1976; Tyrrell, 1976). In a comprehensive study of 21 strains of *E. coli* and *S. typhimurium*, Eisenstark and Perrot (1987) showed that the *recA*

mutation caused greater sensitivity to both H_2O_2 and near-UV radiation than mutations in *katE, F, G, xth* and *nur*.

RecA is also involved in recovery from heat-stress. Sensitivity to mild heat in *E. coli* was increased by both *recA* and *recB* (Mackey and Seymour, 1987) when compared with the sensitivity of the parent strains.

The Role of *polA*

The *polA* mutation has been shown to influence the sensitivity of *E. coli* cells to inactivation by both near-UV radiation and H_2O_2. Anatheswamy and Eisenstark (1977) found *E. coli* mutants carrying *polA* to be slightly more sensitive than *recA, recB* double mutants and considerably more sensitive than *recA* or *recB* single mutants and parent strains to 0.01M H_2O_2. The *polA* strain also showed the greatest number of H_2O_2-induced single-strand breaks (ss-breaks/*E. coli* genome: *polA* 239; *recA, recB* 221; *recA* 178; *recB* 148; wild type 120) which were only repaired to a small extent. Sammartano *et al.* (1986) and Eisenstark and Perrot (1987) also found *polA* mutants to be sensitive to H_2O_2 (50 mM and 16 mM respectively). Eisenstark and Perrot (1987) found that *polA* strains were almost as sensitive as *recA* strains to both near-UV and H_2O_2. Tuveson (1981) also found *polA* strains to be sensitive to near-UV radiation in a nur^+ genetic background. However, in a nur^- background, $polA^-$ and $polA^+$ were indistinguishable in their sensitivity to near-UV irradiation. *Escherichia coli polA* mutants also showed an increased number of 365 nm induced single strand breaks in DNA (wild-type 26 ss-breaks/genome; *polA* 75 ss-breaks/genome) which were not repaired in polymerase-I deficient mutants (Ley *et al.*, 1978).

PolA mutants have also been shown to be sensitive to heat-stress (Mackey and Seymour, 1987) as has been shown for *recA* mutants. *PolA*

strains were more sensitive than *rec* strains to mild heat (52°C). However, if *E. coli polA* cells were heat stressed in buffer then transferred to defined medium (but not rich medium) they were seen to recover to the level of wild-type heat stressed cells suggesting that the damage induced by heat stress was repaired.

The Role of *xthA*

Damage by near-UV radiation and H_2O_2 also appears to involve the Xth gene product, exonuclease III. Demple *et al.* (1983) found that *E. coli* mutants lacking exonuclease III were hypersensitive to H_2O_2 and were killed at 20 times the rate of wild type bacteria and 3 to 4 times the rate of *recA* cells. Demple *et al.* (1986) later showed that the H_2O_2 hypersensitivity of *xth* mutants correlated with the degree of single-strand breakage seen in these mutants as compared to wild type bacteria. Sammartano and Tuveson (1983) showed that the same *xth* mutant used by Demple *et al.* (1983) was also sensitive to broad spectrum near-UV radiation both in exponential and stationary phase cells. The *xth* mutation had little or no effect on inactivation by far-UV radiation.

Another study (Van Sluys *et al.*, 1986) found that whilst the above *xth* strain was very sensitive to 3 mM H_2O_2, it was not sensitive to H_2O_2 generated by an oxidized ascorbate (Vitamin C) solution (ascorbic acid undergoes oxidation in the presence of oxygen and metal ions generating oxygen radicals such as H_2O_2). The ascorbate-copper system induced single-strand breaks. These were suppressed by the presence of catalase supporting the idea that H_2O_2 was participating in the damage of DNA. They concluded that the mechanism of H_2O_2 damage in the two systems was different although the main intermediate (H_2O_2) was the same for both treatments.

The study by Eisenstark and Perrot (1987) also found *xthA* mutants to be sensitive to H_2O_2 and near-UV radiation as compared to wild-type cells. However, *xthA* mutants were not as sensitive as *recA*, *polA* or *katF/nur* mutants. Unlike *recA* and *polA* mutants, lack of endonuclease III (*xth*$^-$) did not sensitize cells to heat-stress (Mackey and Seymour, 1987).

Figure 9 is taken from the study by Eisenstark and Perrot (1987) and serves as a helpful summary for the role of catalase and DNA-repair enzymes in near-UV radiation and H_2O_2 induced damage. It can be seen from this figure that there is considerable correlation between the results seen with near-UV radiation and those seen with H_2O_2. From this figure it can be concluded that protection against near-UV radiation and H_2O_2 is complex and although catalase does have a role in this protection, it may only be a minor one. However, this figure does oversimplify the situation. As discussed above, there is not complete agreement about the relative importance of these various enzymes or of whether or not near-UV irradiation and H_2O_2 challenge share a common damaging mechanism.

It seems likely that near-UV radiation damage and recovery involve mechanisms in addition to those involved in H_2O_2 damage alone. Evidence for this comes from studies in which cells were irradiated with near-UV radiation in the presence of H_2O_2 (Hartman *et al.*, 1979; Anatheswamy *et al.*, 1979). If the effects of the two agents were through the same pathway, they would be expected to act additively not synergistically. This is not seen; for example, the survival of phage T7 is 96% after broad-band near-UV irradiation (5.4×10^4 Jm^{-2}) and 98% after H_2O_2 challenge (0.05%) but when the two treatments are added together survival is greatly reduced to 4.5% (Hartman *et al.*, 1979).

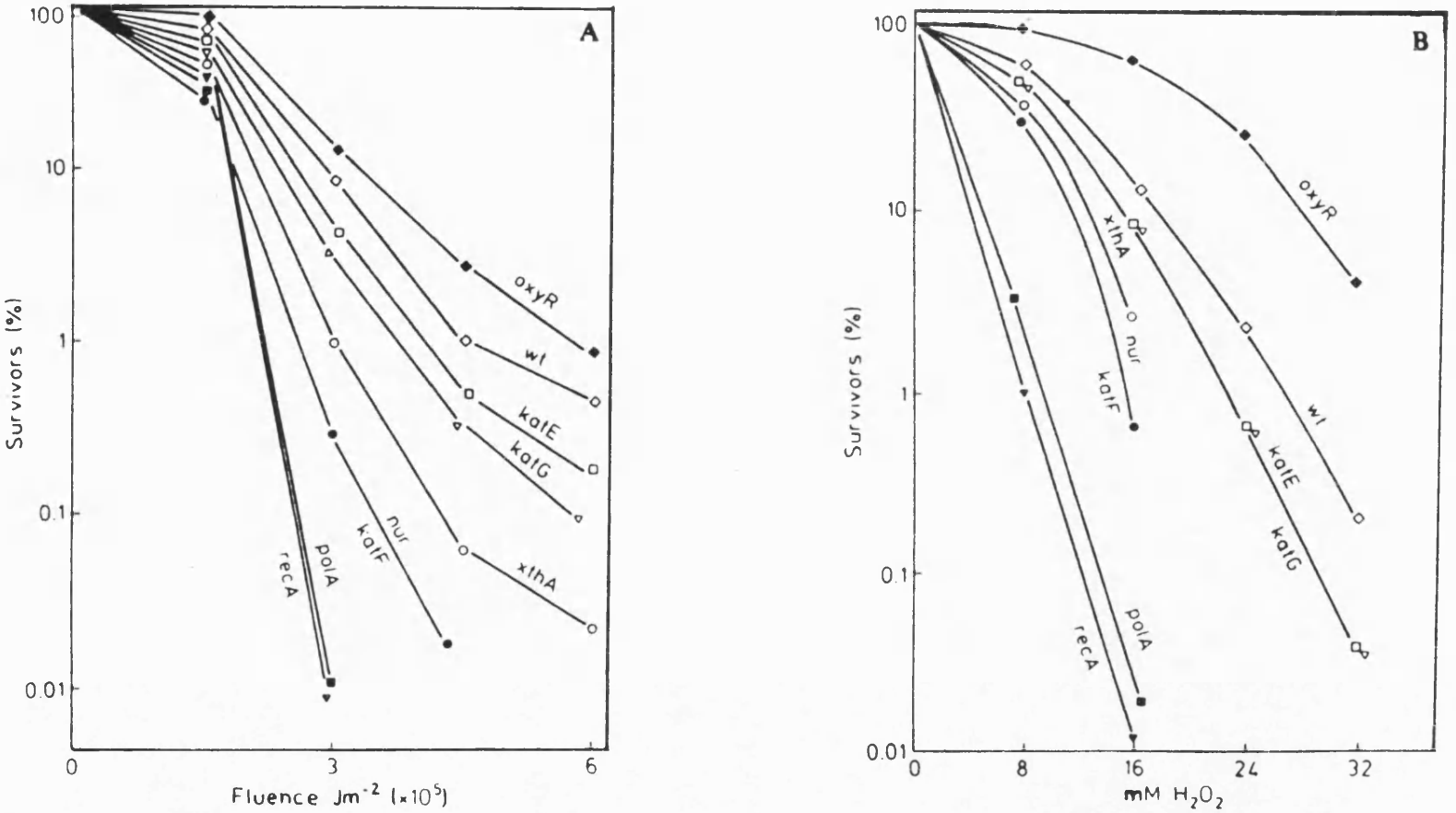


Figure 9: Survival curves for bacterial strains carrying the genetic loci shown,after (A) near-UV irradiation or (B) 10 min exposure to H_2O_2. (Adapted from Eisenstark and Perrot, 1987.)

Problems also arise from different experimental procedures carried out by different workers. The H_2O_2 concentration used in the studies discussed here varies from 200 µM to 50 mM. Imlay and Linn (1986) reported that two modes of H_2O_2 killing of *E. coli* K12 can be distinguished. Mode-one killing was maximal with low concentrations of H_2O_2 (1-2 mM) and required active metabolism during H_2O_2 challenge. *RecA, recB, xthA* and *polA* strains were all sensitive to mode-one killing. Mode-two killing occurred at higher concentrations of H_2O_2 and did not require active metabolism. Imlay and Linn (1986) reported that mode-two killing was frequently seen in response to both H_2O_2 and near-UV radiation treatments. Sensitivity to mode-two killing could be lessened by H_2O_2-mediated induction of the *oxyR* regulon which may increase levels of scavenging enzymes (Christman *et al.*, 1985; and see discussion in next section). In repair-competent strains of *E. coli* the response was a function of total H_2O_2 exposure (H_2O_2 concentration x time). In contrast, repair-deficient strains showed extreme sensitivity to H_2O_2 at concentrations below 3 mM, which was partly reversed at moderate concentrations (3-10 mM) and then increased again for high concentrations (>10 mM). *XthA* and *recA* strains showed this response and although *xthA* was generally more sensitive than *recA*, there was some cross-over at high concentrations. *PolA* was extremely sensitive to H_2O_2 especially at low concentrations.

Inducible Responses to Oxidative Stress

The knowledge that protection against H_2O_2 and oxygen-dependent near-UV radiation damage and its subsequent repair involves both pre-damage mechanisms, such as catalase, and post-damage repair mechanisms, such as those involving the *recA, xthA* and *polA* genes, would imply that this protection is based on a complex oxidative mechanism.

Peters and Jagger (1981) presented evidence for a repair system induced by near-UV radiation in actively growing cultures of *E. coli* K12. This system repaired a major part of the near-UV radiation lethal damage produced in the cells. It was not, however, the same as the far-UV inducible SOS repair system as it was indepedent of the *recA* gene and involved the induction of several new proteins different from the RecA gene product.

A similar inducible pathway in growing *E. coli* cells was reported by Demple and Halbrook (1983). This pathway was induced by exposure to non-lethal levels of H_2O_2 and responded to oxidative DNA damage caused by challenge with a subsequent lethal dose of H_2O_2. Induction of peroxide resistance failed to increase survival of *E. coli* to far-UV radiation, distinguishing the pathway from the *recA*-dependent SOS response. Since the increased resistance of cells to H_2O_2 produced by induction did not correlate with increased catalase or superoxide dismutase levels, Demple and Halbrook (1983) concluded that the induction resulted in increased DNA repair activity. They also observed that this pathway was similar to the adaptive response to alkylating agents in that both repair pathways were induced by non-lethal levels of the respective agents and subsequently the capacity to repair a specific type of lethal DNA damage was increased. Further studies have shown that pathways for inducible repair of near-UV radiation and H_2O_2 damage were related. Sammartano and Tuveson (1985) reported that *E. coli* cells pretreated with a sublethal dose of H_2O_2 prior to inactiviation by near-UV radiation were more resistant to the near-UV radiation challenge than unpretreated cells. This pretreatment did not induce resistance to far-UV radiation. Tyrrell (1985) reported that pretreatment with either H_2O_2 or near-UV radiation protected *E. coli* cells against a subsequent lethal challenge with the other agent. The addition of chloramphenicol to the growth medium during pretreatment prevented the

induced resistance, implying that the induction effect requires protein synthesis.

Similar studies by Mackey and Derrick (1986a) showed that sublethal exposure to mild heat-stress (42°, 45° and 48°C) resulted in *S. typhimurium* cells becoming substantially more resistant to heating at higher temperatures (50-59°). Conversely, cold shock sensitized *S. typhimurium* and *E. coli* cells to oxidative stress (Mackey and Derrick, 1986b). Also, prior exposure of growing cells of *S. typhimurium* to sublethal levels of H_2O_2 abolished sensitivity to oxidative stress following cold shock, implicating the presence of an inducible system protecting against oxidative stress.

These results of Mackey and Derrick (1986a and b) reinforce the proposals of Ames and co-workers who have suggested that resistance to oxidative stress in both prokaryotes and eukaryotes is also linked to acquired heat resistance and that heat-shock proteins are produced in response to both stresses (Lee *et al.*, 1983; Bochner *et al.*, 1984). They reported that in *S. typhimurium* and *E. coli* heat-shock and a variety of oxidative stresses induced the accumulation of AppppA (P^1, P^4-diadenosine-5'-tetraphosphate) (Lee *et al.*, 1984) and a series of related adenylylated nucleotides (Bochner *et al.*, 1984). They proposed that adenylylated nucleotides were alarmones (i.e. signal molecules that alert the cell to the onset of a particular metabolic stress; Stephens *et al.*, 1975) signalling the onset of oxidative stress. They also suggested that particular adenylylated nucleotides may be alarmones for specific oxidative stresses; e.g., ApppGpp (guanosine-3'-diphosphate-5'-adenosine-5'-[P^1, P^3 triphosphate]) for oxidative damage to amino acid biosynthesis.

Heat-shock proteins have also been shown to be induced by ethanol

(Neidhardt *et al.*, 1984), amino acid starvation (Grossman *et al.*, 1985) and certain SOS inducing agents, such as far-UV radiation and nalidixic acid (Krueger and Walker, 1984). This would imply that the induction of heat-shock proteins is part of a general cellular adaptation to stress which can be stimulated by a variety of stresses including oxidants.

Work by Privalle and Fridovich (1987) has shown that the enzyme superoxide dismutase is induced by heat-shock in *E. coli*. They propose that heating increases O_2^- and H_2O_2, producing autoxidations within *E. coli* and that the increased O_2^- production induces biosynthesis of manganese-containing superoxide dismutase. Such an inducible response to O_2^- has been previously reported (Farr *et al.*, 1985). Actively growing *E. coli* cells were shown to be sensitive to plumbagin, a quinone capable of abstracting electrons from electron transfer components and directing the flow of electrons to oxygen to form the superoxide radical. Pretreatment with plumbagin (0.075 mM) protected cells from subsequent treatment with a higher concentration (2 mM) of this O_2^- generator. In this case, however, the induced response was found to be different from the response to oxidative stress caused by exposure to H_2O_2.

These various reports indicate that there is a complex response to oxidative damage and that although overlaps between stresses are seen, there are also differences. Christman *et al.* (1985) confirmed that such a global response was indeed found in bacteria. Christman *et al* (1985) found that *S. typhimurium* became resistant to killing by H_2O_2 and other oxidants when pretreated with nonlethal levels of H_2O_2. During the adaptation to H_2O_2, 30 proteins were induced. They also isolated a class of H_2O_2 resistant mutants, represented by *oxyR1*, which constitutively overexpressed nine proteins normally induced during H_2O_2 adaptation. The *oxyR1* mutants had elevated levels of catalase, peroxidase, manganese-containing

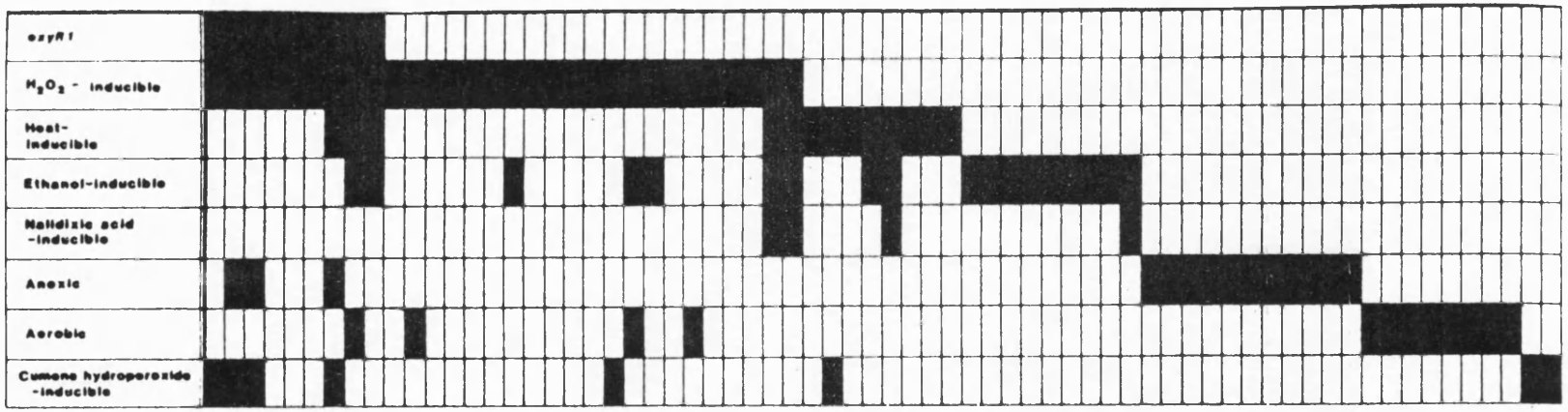


Figure 10: Schematic diagram of the overlaps among stress responses in *Salmonella*. The shaded boxes represent proteins that are induced by the stresses shown in the left-hand column,(taken from Morgan *et al.*, 1986).

superoxide dismutase, glutathione reductase and a novel alkyl hydroperoxide reductase, in addition to three heat-shock proteins. Strains carrying a deletion throught to remove the *oxyR* gene were hypersensitive to H_2O_2 and the nine proteins overexpressed in *oxyR1* could not be induced. Further work by the same group showed that the $\triangle$*oxyR* mutant was also hypersensitive to near-UV radiation (Kramer and Ames, 1987).

Christman *et al.* (1985) proposed that the *oxyR* regulatory network was a major regulon (i.e. a functional unit in which unlinked and individually controlled genes can be coordinated by a common regulatory gene) responding to oxidative stress. This regulon was found in addition to regulons controlling heat-shock (*htp*-controlled), SOS (*lexA*-controlled) and the adaptive response to alkylating agents (*ada*-controlled).

Subsequent work has shown that the proteins induced by H_2O_2 overlap considerably with proteins induced by other stresses although some additional proteins are produced in response to specific inducing agents (Morgan *et al.*, 1986). A schematic diagram of the overlaps among stress proteins in *Salmonella* (taken from Morgan *et al.*, 1986) is shown in fig. 10. Similar results have also been seen in *E. coli* (Van Bogelen *et al.*, 1987). These studies showed that the *htpR*, SOS and *oxyR* regulons could be induced separately, although some agents induce more than one regulon. Van Bogelen *et al.*(1985) also demonstrated that adenylylated nucleotides are not only alarmones for heat-stress since their accumulation also accompanied *oxyR* mediated responses.

GENERAL METHODOLOGY

Bacterial Strains

This study examines the response of *Escherichia coli* to near-ultraviolet radiation. A list of the eleven main *E. coli* strains used in this study, together with their genotypes and source, is given in Table 1. Additional *E. coli* strains will be described in section 3 of the results.

Storage of Bacterial Strains

For long term storage, plastic vials containing cultures of each strain were stored at approximately -196°C in the vapour phase of a liquid nitrogen refrigerator (Union Carbide, Ltd). These were subcultured into nutrient agar stabs which were prepared as follows; nutrient agar (11.2 g, Oxoid CM3 nutrient agar) was added to 400 ml single-glass distilled water in a 1 litre, flat-bottomed flask. The agar was dissolved by boiling and 5 ml of molten nutrient agar was transferred into 8 ml screw-capped glass vials (Wheaton Scientific, New Jersey). The agar stabs were sterilized by autoclaving at 121°C for 15 minutes and allowed to set before inoculating with a sterile straight wire and incubating at 37°C for 24 h. One ml of sterile liquid paraffin B.P. (B.D.H. Ltd) was added aseptically to each stab to limit evaporation. The liquid paraffin was sterilized in 10 ml glass ampoules by heating in a hot air oven at 160°C for 1 hour. These master stabs were stored at room temperature.

For routine use, stabs were taken from the master stab, subcultured onto nutrient agar plates and streaked for single colonies using a sterile wire loop. The nutrient agar for the plates was prepared as described above and sterilized in 1-litre flat-bottom flasks, capped with aluminium foil, by autoclaving at 121°C for 20 minutes. Twenty ml volumes of sterile molten agar were poured into sterile petri dishes (9 cm diameter, Sterilin Ltd, UK) and allowed to set. After inoculation and incubation at 37°C for

Table 1 List of strains used

STRAIN	LAB CODE NO.	RELEVANT GENOTYPE	OTHER GENOTYPE	REFERENCE
K12 AB1157	DV 4	+	F^-, *thr, leu, arg, his, pro, ara, lac, gal, mtl, thi, xyl, tsx, str*^{r}.	Adelburg & Burns (1960) (a)
B/r	DV 132	+	*lon, sul.*	Hill (1964) (b)
K12 JG139 (SR 385)	DV 66	+	F^-, *rha-5, lacZ53, rpsL 151, thyA36, deo (C2?)*	(c)
CSH7	DV 150	+	*lacY, rpsL, thi-1*	Miller (1972) (d)
UMI	DV 151	kat E1	*lacY, rpsL, thi-1*	Miller (1972) (d)
SR 833	DV 82	+	B/r *lac*, *val*^{ts}, P_1 restrict	Ramabhadran & Jagger (1976) PNAS 73 59 (c)

Table 1 (continued)

STRAIN	LAB CODE NO.	RELEVANT GENOTYPE	OTHER GENOTYPE	REFERENCE
SR 834	DV 83	relA	B/r *lac*, *val*^{ts}, P_1 restrict	Ramabhadran & Jagger (1976) PNAS 73 59 (c)
NF 161	DV 179	+	F^-, *metB1*, *argA52*, *spoT1*, λ^R?, λ^-	Laffler & Gallant (1974) (e)
NF 162	DV 180	rel A	F^-, *metB1*, *argA52*, *spoT1*, λ^R?, λ^-	Laffler & Gallant (1974) (e)
SR 246	DV 51	PolB100	F^-, *thyA*, *thyR*, *lys*, *lacZ*, *su*$^-$, *str*^{r}	Constructed at Stanford University (e)
SR 362	DV 65	uvrA, uvrB recA, phr	F^-, *leu*, *thyR*, *met*, *rha*, *lacZ*, *str*^{r}	Constructed at Stanford University (c)

(a) obtained from Dr B. A. Bridges whilst at MRC Radiobiology Unit, Harwell, UK
(b) obtained from Dr N. Gillies, The Middlesex Hospital Medical School, London, UK
(c) obtained from Prof. K. C. Smith, Stanford University, USA
(d) obtained from Dr P. C. Loewen, University of Manitoba, Canada
(e) obtained from Dr B. Bachmann, *E. coli* Genetic Stock Centre, Yale University, USA

24 h, plates were sealed with adhesive tape and stored, inverted, at 4°C for up to 4 weeks. Fresh plate cultures were obtained from the master stab as required. The growth requirements of strains were routinely tested on defined media to confirm their phenotypes.

Glassware

Glassware was washed with tap water, rinsed three times in distilled water and dried overnight in an oven. No detergent was used in the washing procedure in order to protect the bacterial membrane from possible damage caused by residual detergent present during experiments, as has been shown by Moss and Smith (1981).

Glass containers, such as culture flasks, that required sterilization were capped with aluminium foil and sterilized by heating in a hot air oven at 160°C for a minimum of one hour. Glass pipettes were packed into D.H.S.S. specification bags (J. Dickenson and Sons, Ltd) before sterilizing by autoclaving at 121°C for 15 min.

Liquid Media Used for the Growth of Bacterial Strains

Organisms were grown in either complex or defined growth media.

a) Preparation of Complex Medium

Unless otherwise stated, the complex growth medium used was nutrient broth. This was prepared by dissolving nutrient broth (13 g, Oxoid CMI) in 1 litre of distilled water. This was then sterilized as 100 ml volumes in 150 ml screw-capped glass bottles by autoclaving at 121°C for 15 minutes. The final pH of the media was 7.2-7.4.

b) Preparation of Defined Media

The defined media used was composed of M9 salts solution (Anderson, 1946) with the addition of the growth requirements for the particular bacterial strain in use. The M9 salts solution was made up as two concentrates, M9A ad M9B;

M9A	NH_4Cl	50 g
	$MgSO_4.7H_2O$	10 g
	water to 1 litre	

This is a 50x concentrate.

M9B	KH_2PO_4	37.5 g
	$Na_2HPO_4.2H_2O$	75 g
	NaCl	6.25 g
	water to 1 litre	

This is a 12.5x concentrate.

The chemicals were 'Analar' grade from B.D.H. Ltd. The two concentrates were sterilized separately as 250 ml volumes in 500 ml screw-capped glass bottles by autoclaving at 121°C for 20 minutes.

Defined medium was prepared by mixing M9A (20 ml) and M9B (80 ml) with the volumes of water shown in Table 2. This solution was distributed equally among ten 150 ml screw-capped glass bottles and sterilized by autoclaving at 121°C for 15 min.

Sterile growth supplement solutions were added aseptically to the sterile M9 salts solution as required. The growth supplements added for each particular strain are also shown in Table 2.

Table 2

Composition of Defined Media

STRAIN	M9A (ml)	M9B (ml)	DISTILLED WATER (ml) FOR BATCH OF 10x100ml	VOL (ml) M9A +M9B+WATER PER 100 ml BATCH	40% GLUCOSE (ml)	1 mg/ml THYMINE (ml)	1mg/ml THIAMINE (ml)	0.1M AMINO ACID SOLUTIONS (ml)
AB1157	20	80	835.0	93.50	1.0	-	0.5	1.0 each leu, pro, arg, his, thr.
B/r	20	80	889.5	98.95	1.0	-	0.05	-
SR 385	20	80	879.5	97.95	1.0	1.0	0.05	-
CSH7	20	80	885.0	98.50	1.0	-	0.5	-
UM1	20	80	885.0	98.50	1.0	-	0.5	-
SR833	20	80	869.5	96.95	1.0	-	0.05	1.0 each val, isoleu

Table 2 (continued)

STRAIN	M9A (ml)	M9B (ml)	DISTILLED WATER (ml) FOR BATCH OF 10x100ml	VOL (ml) M9A +M9B+WATER PER 100 ml BATCH	40% GLUCOSE (ml)	1 mg/ml THYMINE (ml)	1mg/ml THIAMINE (ml)	0.1M AMINO ACID SOLUTIONS (ml)
SR834	20	80	869.5	96.95	1.0	-	0.05	1.0 each val, isoleu
NF161	20	80	869.5	96.95	1.0	-	0.05	1.0 each met, arg
NF162	20	80	869.5	96.95	1.0	-	0.05	1.0 each met, arg
SR246	20	80	869.5	96.95	1.0	1.0	0.05	1.0 lys
SR362	20	80	859.5	95.95	1.0	1.0	0.05	1.0 each leu, met

Preparation of Stock Solutions of Growth Supplements

a) Glucose

Glucose was made up as a 40% w/v stock solution by dissolving D-glucose (40 g, 'Analar' grade, B.D.H. Ltd) in 100 ml distilled water. This solution was sterilized through a 25 mm swinnex unit (Millipore U.K. Ltd, London), previously sterilized by autoclaving at 121°C for 15 min, fitted with a 0.2 µm pore size membrane filter (Sartorius membrane filters, GMBH, West Germany). The solution was stored in a sterile 150 ml screw-capped glass bottle at 4°C. The solution was diluted 100-fold to give a final concentration of 10 µg/ml.

c) Thiamine

A stock solution of thiamine was made up as a 1 mg/ml solution by dissolving thiamine HCl (100 mg, Sigma) in 100 ml distilled water. This was sterilized by membrane filtration as described above for glucose. The concentrate was stored in a sterilized 150 ml screw-capped glass bottle at 4°C and protected from light by wrapping the bottle in aluminium foil. This stock solution was diluted either 1 in 200 for thiamine requiring strains giving a final concentration of 5 µg/ml, or 1 in 2000 for strains with no thiamine requirement to give a final concentration of 0.5 µg/ml.

d) Amino Acids

All amino acid solutions were prepared as 0.1 M solutions in distilled water. All chemicals were obtained from Sigma Chemical Company. The solutions were sterilized by membrane filtration and stored as described above for glucose. These concentrates were diluted 100-fold to give a final concentration of 10^{-3} M.

Preparation of M9 Salts Solution for Use as a Diluent

M9 salts solution was also used as a diluent, to wash cells and as a buffer in which to resuspend cells for treatment. This solution was prepared by mixing 180 ml M9A and 720 ml M9B and making the volume up to 9 litres with distilled water. This was sterilized as 250 ml volumes in 300 ml conical flasks, capped with aluminium foil, by autoclaving at 121°C for 15 minutes. The final pH of this solution was 6.9.

Method Used for Growth of Bacterial Strains

A 250 ml flask containing 100 ml prewarmed growth medium was inoculated with a loopful of culture taken off an agar plate. This was incubated in a shaking water bath (Gallenkamp water immersion bath, set at 37°C $\pm$ 0.5°C, shaking at 100 cycles per minute, safety cut-out set at 50°C) for 16 h (i.e. overnight). The culture was aerated by drawing a standardised volume of air through the culture vessel. Atmospheric air was drawn through a wool filter by a piston pump (40 watt, Reciprotor, Edwards High Vacuum Ltd, Sussex, UK) into a 5 litre bell jar. Air passed from the bell jar into the culture vessel at 10 ml per minute. The flow rate of air was regulated by a flow meter (Rotameter, Croydon, UK). Air was sterilized by passing through a 25 mm membrane filter with pore size 0.45 µm (Sartorius membrane filters, GMBH, West Germany) contained in a 25 mm Swinnex unit (Millipore UK Ltd, London) positioned immediately before the entrance to the culture vessel.

A secondary culture was obtained by transferring 1 ml of the 16 h, primary culture to 100 ml prewarmed growth medium and incubating for 24 h in the same manner as described above. Stationary phase cells were obtained from this 24 h secondary culture. Exponential phase cells were obtained by inoculating 100 ml prewarmed growth media with 1 ml of the 24 h

secondary culture and incubating for an appropriate length of time. This length of time was determined from a growth curve for the particular strain, growth medium and conditions being used. A typical growth curve is shown in fig. 1, Appendix A, giving the change in optical density with time for *E. coli* K12 AB1157 grown in M9 defined media. The optical density of the bacterial culture was measured in a plastic cuvette in a Unicam SP600 spectrophotometer, set at 470 nm, using an appropriate blank, in this case M9 defined medium. Samples with optical densities greater than 0.35 were diluted with the appropriate medium, since samples with optical density values greater than this did not adhere to the Beer-Lambert relationship.

Harvesting of Bacterial Strains

Cells were harvested using a filtration method. The apparatus used was a negative pressure Sartorius filtration Unit (Sartorius, GMBH, West Germany). The unit was packed into a DHSS specification bag (J. Dickenson and Sons Ltd) and sterilized by autoclaving at 121°C for 15 min. Sartorius 25 mm diameter membrane filters with a pore size of 0.45 µm were packed separately between 50 mm diameter filter papers (Whatman No. 1) in DHSS specification bags and sterilized by autoclaving at 121°C for 15 min. For filtration of cells, a membrane filter was placed aseptically on the sintered glass supporting bed of the filtration unit. The filter was washed three times prior to and three times after filtration of the cells with 10 ml volumes of M9 salts solution. A known volume of cell suspension was filtered, usually 1.0 ml for stationary phase cells and 10.0 ml for exponential phase cells. The filter was then aseptically transferred to a sterile 100 ml beaker containing 10 ml M9 salts solution. The cells were resuspended by gentle agitation and diluted to give the appropriate cell density, normally 10^7 colony forming units (CFU) per ml.

Method Used to Assess Viability

The viability of cells was assessed by their ability to form colonies on solid media. Samples were serially diluted before being spread on solid agar plates and incubated. The following equipment was used in this procedure.

a) Pipettes

Cell suspensions were prepared and samples taken using two Gilson pipettes (Gilson Medical Electronics, France) with volume ranges of 0.2-1.0 ml and 1.0-5.0 ml. The accuracy of the volumes delivered by the pipettes was determined by weighing volumes of distilled water. Glass distilled water was delivered under experimental conditions with the pipette being reset and a fresh tip fitted on each occasion. The results are presented in Table 3 and show that the coefficient of variation obtained was not greater than 1.37% and the difference as a percentage of the theoretical weight was a maximum of 0.60% for the 0.2 ml volume. The plastic changeable tips for the Gilson pipettes were washed and boiled in distilled water, dried, packed into DHSS specification bags and sterilized by autoclaving at 121°C for 15 min.

b) Dilution Tubes

Samples were diluted in M9 salts solution and the serial dilutions were performed in 6" x 0.75" glass test-tubes fitted with Oxoid metal caps. The tubes were washed and rinsed as described for glassware and the caps were boiled in distilled water. After drying, the tubes were capped and sterilized in a hot air oven at 160°C for a minimum of 1 h.

Table 3 Accuracy of Gilson Pipettes

Volume (ml)	Pipette	Mean (g)	Standard deviation from mean	Coefficient of variation	Differences as % of theoretical mean
0.2	P 1000	0.1988	2.7206×10^{-3}	1.368	0.60
0.5	P 1000	0.4982	2.8479×10^{-3}	0.572	0.36
3.8	P 5000	3.7936	2.8035×10^{-2}	0.739	0.17
4.5	P 5000	4.5132	2.6316×10^{-2}	0.583	-0.29

Each volume of distilled water was weighed 15 times (n=15) using an analytical balance at constant humidity.

c) Spreaders

Spreaders were made from 5" lengths of glass rod with a 90° bend two thirds along their length. Spreaders were boiled in distilled water and rinsed as for glassware then dried as above. Batches of 25 spreaders were placed in a 250 ml beaker, capped with aluminium foil and sterilized in a hot air oven at 160°C for a minimum of 1 h.

The serial dilution was performed by transferring a 0.2 ml sample of the test cell suspension to 3.8 ml M9 salts solution in a dilution tube. This was followed by a series of 10-fold dilutions each of which was achieved by transferring 0.5 ml of the previous dilution to 4.5 ml M9 salts solution. The cell suspension was thoroughly mixed using a whirlimixer (Fisons Ltd) after each dilution. Since errors may be introduced by failing to achieve a completely homogenous suspension, an experiment to test homogeneity was carried out on a representative experimental suspension. The results shown in Table 4 indicate that errors introduced in producing a homogenous suspension, which includes pipetting and dilution errors, constitute a major factor in the overall experimental error with a coefficient of variation of 3.87%.

When the serial dilution had been carried out, a 0.2 ml sample of the required dilution was pipetted onto the surface of an agar plate and spread over the surface using the sterile glass spreaders. A minimum of two plates, normally three plates, was used for each dilution plated. A range of dilutions were sampled for each point so as to obtain an optimum number of colonies for counting i.e. between 30 and 300 colonies per plate.

Table 4 Homogeneity of Suspension

Series	Plate Count				Mean viable count x 10^7 / ml.
1	132	154	130	161	1.44
2	128	139	143	149	1.40
3	153	150	148	141	1.48
4	150	151	139	128	1.42
5	132	144	131	139	1.37
6	128	145	146	140	1.40
7	150	152	148	139	1.47
8	150	153	149	145	1.49
9	128	127	133	138	1.31
10	143	145	150	138	1.44

Overall mean viable count 1.42×10^7
Standard deviation 5.49×10^5
Coefficient of variation 3.87%

The 10 series were obtained by performing ten 10^5 serial dilutions on a single *E. coli* K12 AB1157 bacterial suspension

Plates were incubated, inverted, at 37°C for either 48 h for complex media or 72 h for defined media plates. The number of viable bacteria in the original suspension was calculated from the mean number of colonies per plate and the dilution factor.

Solid Media Used for Growth of Bacterial Strains

Cells were either grown on complex plating medium or defined plating medium.

a) Complex Media

Three complex media were used in this study; Oxoid nutrient agar, tryptone agar and yeast extract nutrient agar.

Oxoid nutrient agar (NA) was described under the storage of bacterial strains section.

Tryptone agar (TA) was prepared by adding sodium chloride (4 g, 'Analar' grade B.D.H. Ltd), tryptone (4 g, Bacto) nutrient broth (2.3 g, Bacto) and agar technical number 3 (9.6 g, Oxoid) to 800 ml distilled water in a 1 litre flat-bottomed flask. When the powders had dissolved, the flask was capped with aluminium foil and sterilized by autoclaving at 121°C for 20 min. Twenty ml volumes of molten agar were aseptically poured into sterile, 9 cm diameter, plastic petri dishes (Sterilin Ltd, UK).

Yeast extract nutrient broth agar (YENB agar) was prepared by dissolving nutrient broth (6.4 g, Difco), yeast extract (6 g, Difco) and agar technical number 3 (9.6 g, Oxoid) in 800 ml distilled water in a 1 litre flat-bottomed flask. The media was sterilized and poured as described above.

b) Defined Medium

Defined plating medium was occasionally used. This was composed of M9 defined growth medium for a particular strain, as detailed in Table 2, solidified with 1.2% agar technical number 3 (Oxoid). The agar was added to and sterilized with the M9A, M9B, water mixture. Sterile growth supplements were added aseptically to the sterile molten agar when it had cooled to approximately 60°C. The defined medium was mixed by rotating the flask and plates were poured as detailed above.

All plates were stored at 4°C in the dark for upto 7 days. Immediately before use, surface moisture was removed by drying the plates in an open, inverted position at 37°C for 60 mins.

Irradiation Procedures

All irradiation experiments were performed in a dark room with illumination from red fluorescent tubes (Atlas Ltd, 80W) which do not emit light at wavelengths below 540 nm. Three radiation sources were used; one to produce broad-band near-UV radiation, one to produce monochromatic radiation of varying wavelength and one to produce 254 nm far-UV radiation. The three sources are described in detail below together with general irradiation procedures and determination of fluence rate for each source.

1) Broad-Band Near-UV Radiation

Source

Broad-band near-UV radiation was provided by Black-light Blue (BLB) fluorescent lamps. The apparatus is shown diagrammatically in fig. 11. This consisted of six Sylvania F15T8 Black-light Blue lamps (Sylvania Electrical Products Inc., Danvers, Mass., USA) mounted as two vertical

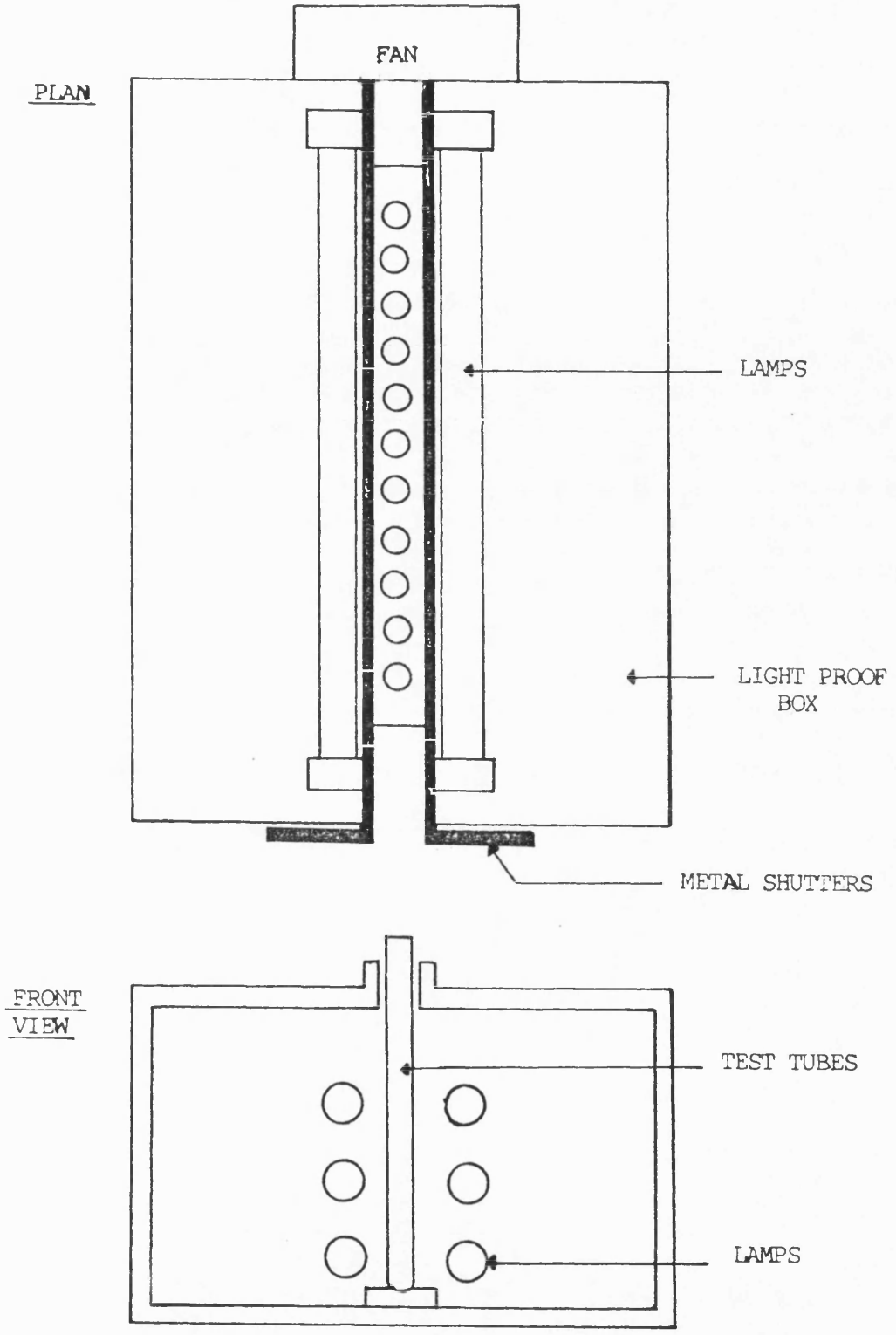


Figure 11: Diagram of near-ultraviolet light source.

banks of three lamps. The emission spectrum of the lamps, shown in fig. 12, is from 320 to 405 nm with an emission peak at 355 nm. The lamps were cased in a black, wooden, light-proof box. During use, the lamps were cooled by means of a fan situated at the rear of the apparatus. Samples were maintained at 28°C±1°C during irradiation. Twelve pyrex test-tubes (Oxoid) could be held vertically between the lamps and irradiated simultaneously. The test-tubes were 4 cm from the front surface of the lamps and 1.5 cm apart from each other. Exposure could be started and stopped by movement of metal shutters between the lamps and the test-tubes. New lamps were run for 100 h before use so that a steady output was achieved.

General Near-UV Irradiation Procedure

Before each experiment, the BLB lamps were allowed to "warm-up" for 20 minutes. The fluence was determined as described in the near-UV radiation dosimetry section below. Normally 5 ml volumes of cell suspension were irradiated. Samples were placed as centrally as possible in the lamp-box depending on the number of samples being irradiated together. The outer three positions at either end of the lamp-box were not used for samples. Any positions not in use were filled with test-tubes containing 5 ml M9 salts solution, this was to help keep reflected light constant. Samples were mixed during irradiation by bubbling filtered, humidified air via a sterile Pasteur pipette into the cell suspension. Samples were left in position for 5 min with the shutters closed to allow the samples time to reach ambient temperature. Exposures were timed with a stopclock. Samples were removed at the appropriate times, serially diluted as required and assessed for viability.

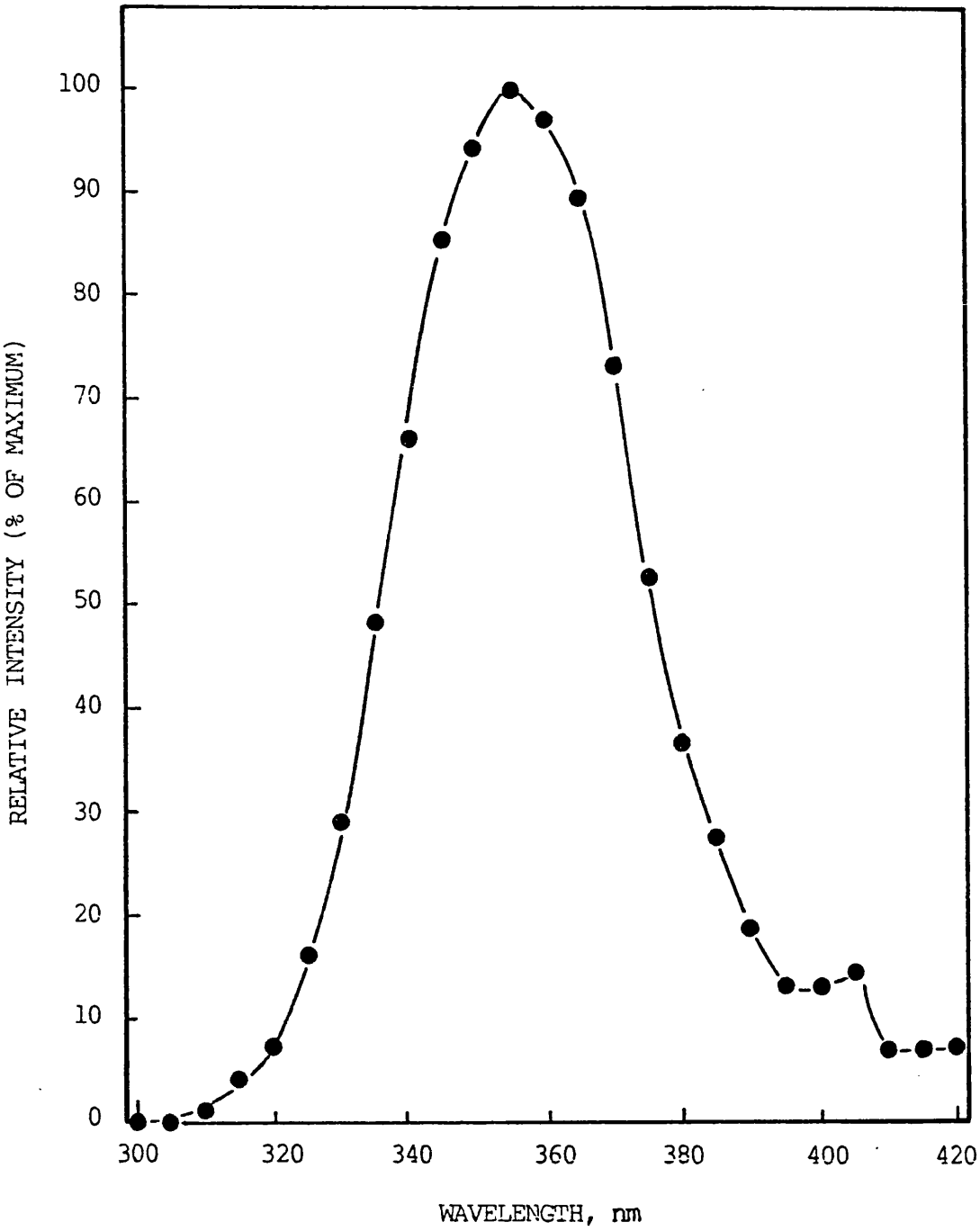


Figure 12: Emission spectrum of near-ultraviolet Black-light-Blue fluorescent light source.

Determination of Fluence Rate for Broad-Band Near-UV Radiation

Determination of the fluence rate of the BLB lamps by use of a thermopile calibrated by chemical actinometry was not possible with this apparatus for two reasons. Firstly, it was not possible to insert the thermopile between the two banks of the BLB lamps so that the entire field of radiation could be detected. Secondly, if chemical actinometry was used (see actinometry section below, under monochromatic fluence rate determination), the length of exposure required to give a millivolt to fluence rate ($Jm^{-2}s^{-1}$) conversion factor within the limits of the calibration curve would necessitate very short exposure which would not allow accurate measurement. Therefore, an approximate fluence rate was determined by comparing the sensitivity of a bacterial strain to published data. The bacterial strain used for dosimetry was *E. coli* K12 SR362 (*uvrA, uvrB, recA, phr*) which was compared with the published 365 nm radiation sensitivity of *E. coli* K12 AB2480 (*uvrA, recA*) (Brown and Webb, 1972). Two assumptions were made when making this comparison, firstly, that these two bacterial strains would show similar near-UV radiation sensitivity. Both strains carry *uvrA* and *recA* and, although AB2480 is not *phr*, Brown and Webb (1972) carried out the irradiation at 0°C where no photoreactivation can occur. Secondly, it was necessary to assume that the broad-band near-UV radiation used in this study could be approximated to 365 nm monochromatic radiation. Although survival curves have been published using BLB lamps as the near-UV radiation source, differences in the phosphors used in the lamps may lead to variations in their emission spectra (Forbes *et al.*, 1976) and direct comparisons cannot be made. Since the comparison of broad-band and monochromatic radiation is necessary, and justifiable if the emission spectrum of the BLB lamps (fig. 12) is considered, it does not take into account the possibility of wavelength interactions occuring with broad-band radiation. This may result in errors in the estimation of the

fluence rate of the BLB lamps. It must be stressed that this method is used to give a comparison with published data and, as such, is not strictly dosimetry.

The survival curves for SR362 after broad-band near-UV irradiation and AB2480 after 365 nm monochromatic radiation (from published data) are shown in fig. 13. Data obtained by Kelland (1984, PhD Thesis) for SR362 using the BLB source described in this study, are also shown in fig. 13. The fluence rate for the BLB lamps was calculated by comparing the time of exposure to broad-band near-UV radiation that reduced survival to 10%, to the 365 nm radiation fluence required to reduce survival to the same level. This was found to be 1.71×10^{3} $Jm^{-2}sec^{-1}$, compared to 1.08×10^{3} $Jm^{-2}sec^{-1}$ estimated by Kelland (1984, PhD Thesis). The differences seen between these two estimates may be due to differences in the lamps being used and differences in the treatment of the bacterial suspensions used. Kelland (1984, PhD Thesis) carried out a second comparison to estimate the fluence of the broad-band near-UV radiation source used in this study, using *Bacillus subtilis* UVSSP 42-1 spores and comparing their sensitivity to published data by Tyrrell (1978). This method gave a slightly lower fluence rate of 9.58×10^{2} $Jm^{-2}sec^{-1}$.

Since this biological comparison dosimetry method gives only an approximate fluence rate, all survival curves in this study that use the BLB lamps as a radiation source are expressed in terms of the time of exposure (in minutes). Survival curves for SR362 were routinely determined and these showed that the fluence rate for the BLB lamps did not alter significantly until they had reached 1000 h of use. After this point, the fluence rate began to decrease slightly and new lamps were installed.

Once an approximate fluence rate had been established, survival curves for *E. coli* K12 AB1157 were determined for the six central irradiation

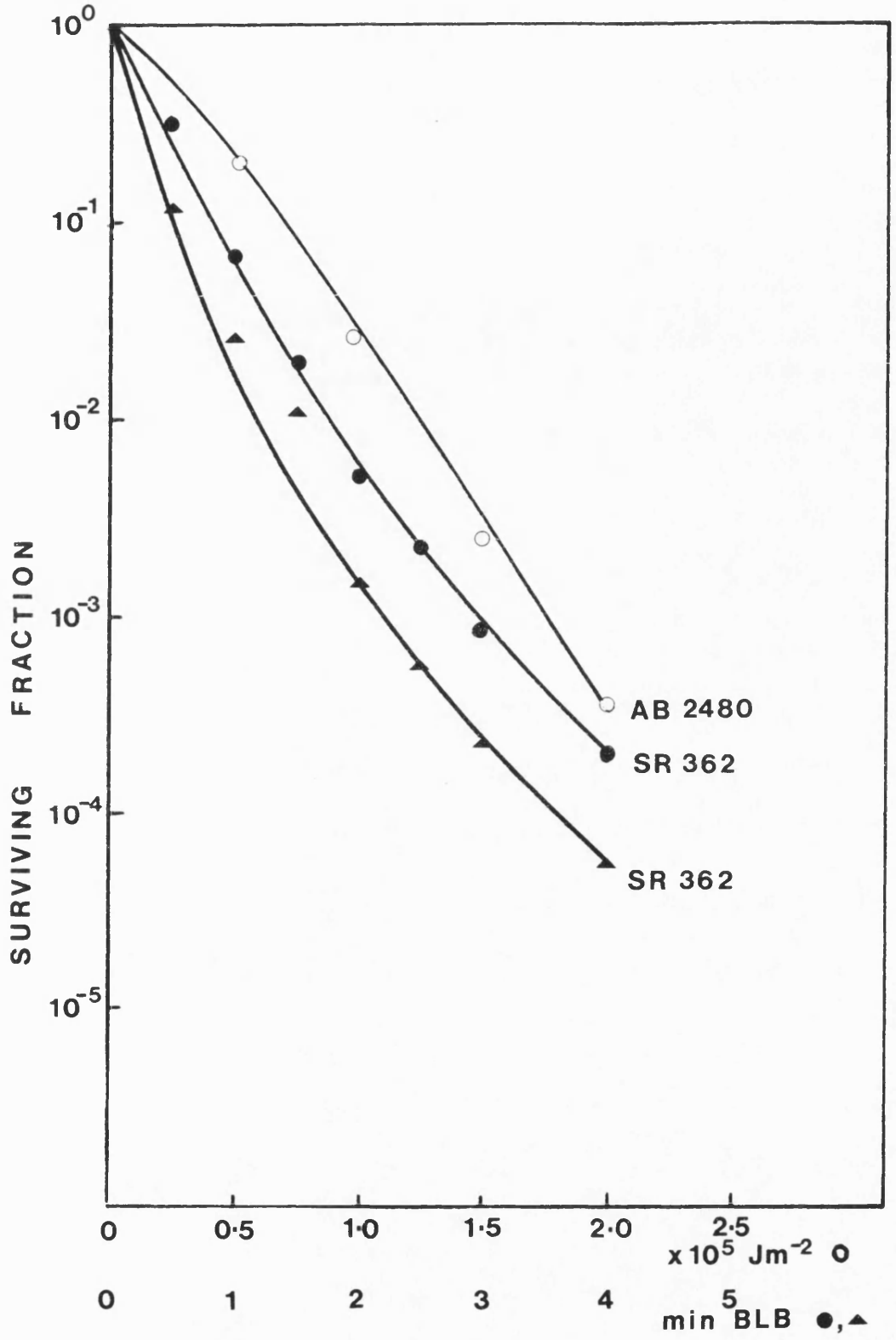


Figure 13: Biological dosimetry comparisons for BLB light source: this study (▲); Kelland, 1984, PhD Thesis, (●); 365 nm radiation source, Brown and Webb, 1972, (o).

positions of the lamp box to ascertain that these positions were receiving equal fluence rates. Bacterial suspensions obtained from a single stationary phase culture were irradiated simultaneously giving the survival curves shown in fig. 14. This shows that the six suspensions placed in positions 4 to 9 of the lamp box (positions numbered 1-12 from rear to front) received approximately equal fluence rates.

2) Monochromatic Radiation

Source

Monochromatic radiation was obtained from a Bausch and Lomb SP200 super pressure mercury lamp (Bausch and Lomb, Rochester, New York, USA). This was used in conjunction with a Bausch and Lomb high intensity grating monochromator. The monochromator was fitted with a UV-visible diffraction grating (1350.0 lines per mm, stated reciprocal dispersion 6.4 nm/mm and entrance and exit slits 2.68 mm and 1.5 mm respectively). A range of wavelengths from 200 nm to 800 nm could be obtained using this apparatus.

The monochromator was aligned along an Ealing Beck optical bench and a focused beam of required wavelength was obtained using lenses and stray light filters. The general arrangement of the components along the optical bench is shown in fig. 15.

Components of Optical Arrangement Shown in Figure 15

The mercury UV source was a 200W super pressure mercury vapour lamp with a fused silica envelope. Unfiltered light from the lamp passed through a Quartz collective lens to the Bausch and Lomb monochromator described above. These three components were cased in a wooden, light-proof box to minimize excessive stray light. The shutter was an iris

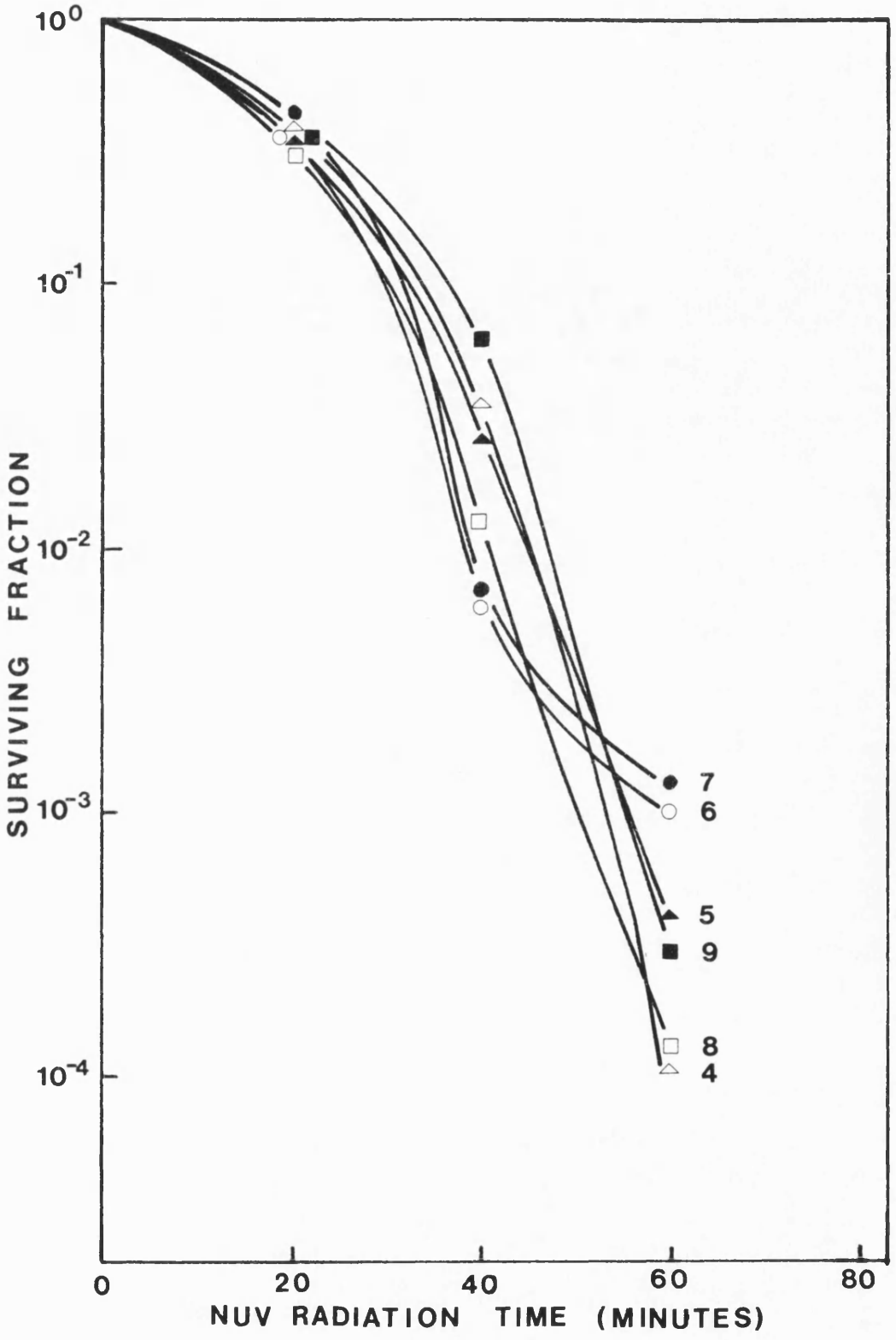


Figure 14: Survival curves for *E. coli* K12 AB1157 after broad-band near-UV irradiation at various positions (as numbered) in the BLB lamp-box.

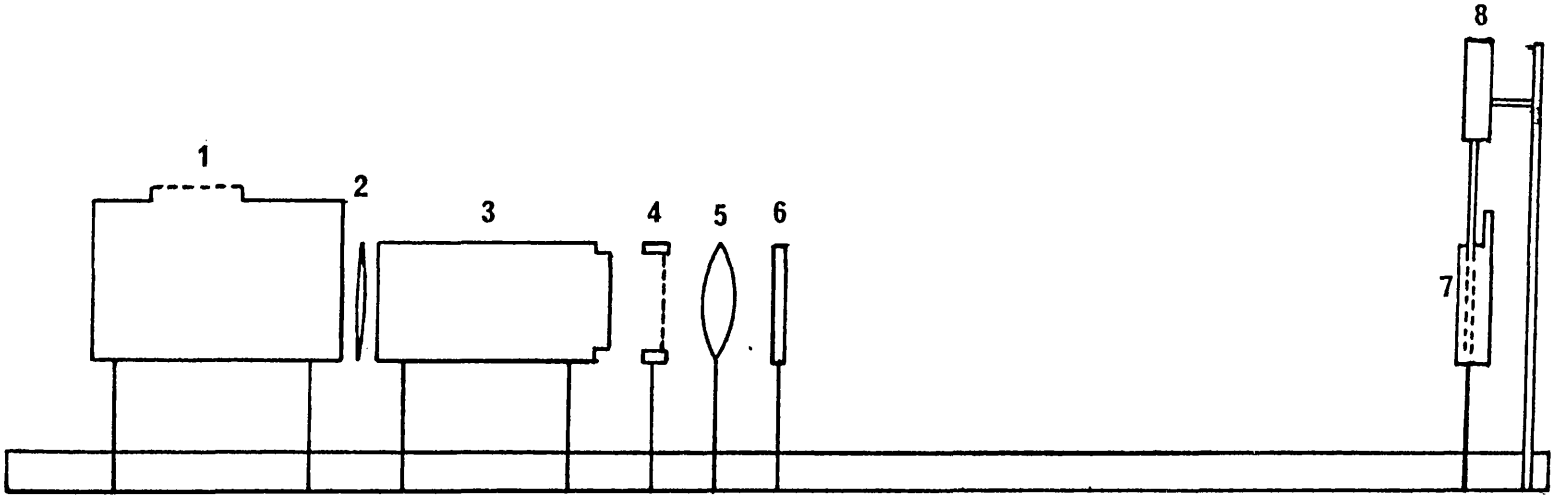


Figure 15: Diagram of apparatus for monochromatic UV irradiation of cells in suspension.

1. Mercury UV source (SP200)
2. Quartz Collective Lens
3. Monochromator
4. Shutter
5. Focusing Lens
6. Stray Light Filter
7. Irradiation Cuvette
8. Stirrer

camera shutter (G.B. Kershaw, 630, 2 cm aperture) fitted with a cable release. The focusing lens was a 40 mm diameter spectrosil biconvex lens of focal length 55 mm. This procduced an inverted, magnified image of the exit slit of the monochromator on the irradiation cuvette. Stray light filters were placed between the lens and the irradiation cuvette. Details of stray light filters are shown in Table 5 and fig. 16. It is important that filters are employed when near-UV monochromatic radiation is being used so that the amount of shorter wavelength radiation being transmitted is reduced to a minimum. The high energy properties of short wavelength radiation could cause significant biological damage which would lead to a higher degree of inactivation than that caused by the near-UV radiation on its own.

The irradiation cuvette was a jacketed 10 mm internal width 10 mm pathlength quartz cuvette. Normally a 3 ml sample of cell suspension was irradiated. The sample was mixed during irradiation by means of a quartz paddle. This was rotated by a laboratory stirrer (Stanhope Seta Ltd) stirring at approximately 1200 rpm.

Irradiations were carried out at room temperature in this study. However, temperature control was possible with this apparatus and achieved by circulating a 50% ethylene glycol solution through the jacketted cuvette. The ethylene glycol solution could be cooled to the required temperature, frequently 0°C in the literature, using a refrigeration unit and was circulated through rubber tubing and the jacket of the cuvette with a peristaltic pump.

Since the output from the lamp fades as the lamp ages, they were replaced every 100 h. New lamps were run for approximately 2 h before use to achieve a steady output.

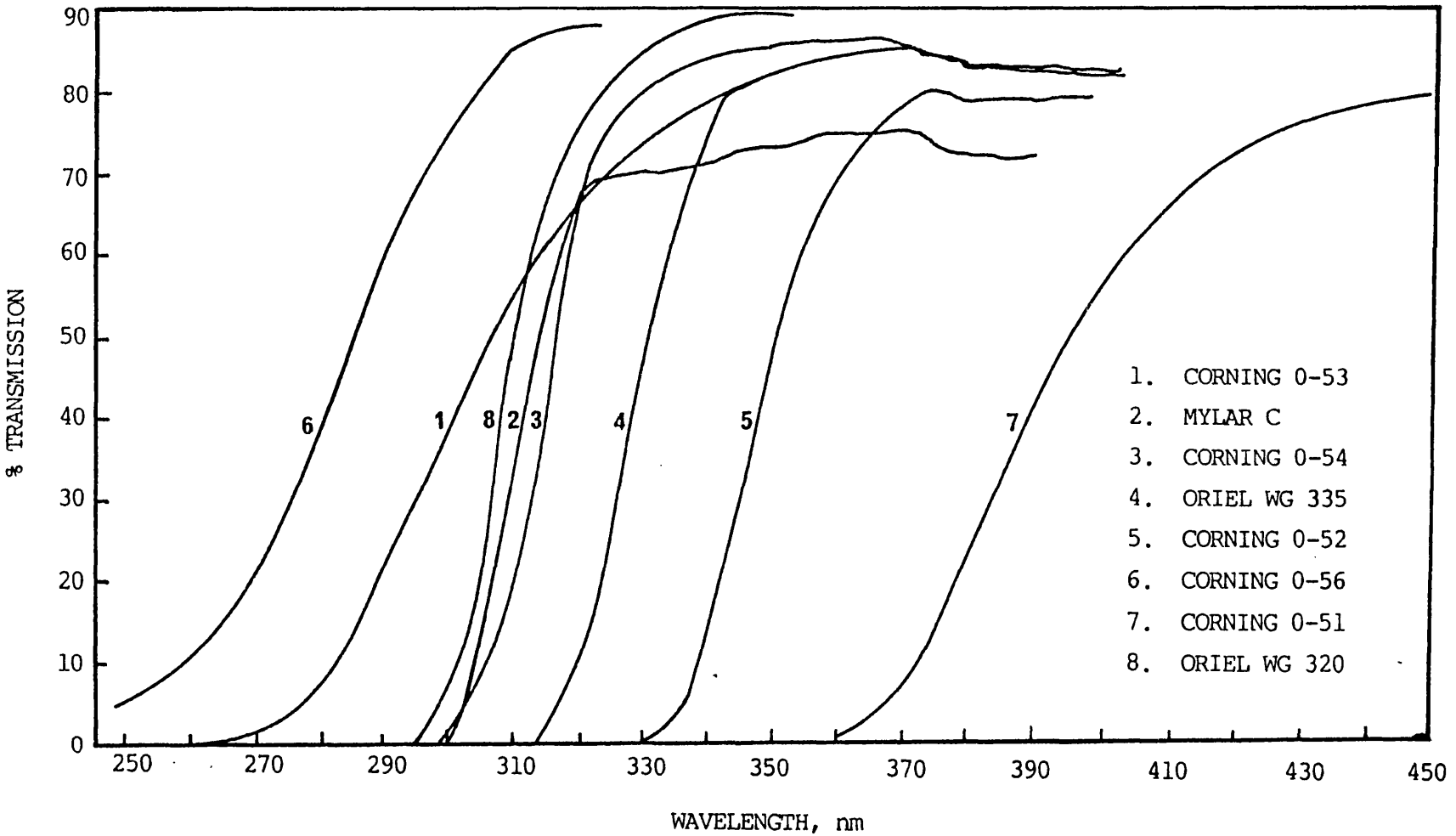


Figure 16: Transmission characteristics of stray light filters.

	Wave-length (nm)	SOURCE	STRAY LIGHT FILTER	Wavelength 1% transmission on Low side (nm)	% transmission at designated wavelength	Band width at 50% maximum (nm)	Approx. fluence rate $Jm^{-2}sec^{-1}$
*	254	Penray	G-275	252	92 without filter (Child's 1962)	4.0	0.05
*	254	B&L	-	-	95 with filter	12.0	0.98
	260	"	-	-	-	12.0	0.22
	270	"	-	-	-	12.0	0.24
	280	"	-	-	-	12.0	0.73
	290	"	CORNING 0-56	240	60	13.0	2.2
	300	"	CORNING 0-53	268	47.9	10.0	4.5
	305	"	CORNING 0-53	268	56.2	13.5	7.0
	310	"	CORNING 0-54	302	20	8.5	7.0
*	313	"	Mylar 2.5 μm	301	54	7.0	25
	317	"	WG 320	296	72.4	9.5	20
	325	"	CORNING 0-54	302	84	12.5	16
	328	"	WG 335	314	50	13.0	18
*	334	"	WG 335	314	68	7.25	14
*	365	"	CORNING 0-52 (HALF THICKNESS)	332	75	7.0	60
*	365	Oriel	"	330	72	10.0	95
*	405	B&L	CORNING 0-51	362	66	7.0	160
*	405	Oriel	"	360	62	10.0	210

* denotes mercury resonance line.

Table 5: Details of stray light filters, band widths and approximate fluence rates used in monochromatic irradiation experiments.

General Irradiation Procedure for Monochromatic Radiation

The monochromator lamp was allowed to "warm-up" for 30-60 min before use. The cuvette was aligned along the optical bench and the height and distance of the cuvette from the radiation source were adjusted so that the front face of the cuvette was completely within the field of illumination. A thermopile was then moved into the radiation beam and the fluence rate calculated as described below (see dosimetry section below). The cuvette was then moved back into position. The cuvette and stirrer were sterilized immediately before use by 15 min exposure to 254 nm radiation from a Penray lamp (see below) placed 3 cm in front of the cuvette. A 3 ml volume of cell suspension was irradiated in the sterile cuvette. Exposure was timed with a stopclock and samples taken were serially diluted and assessed for viability as described before.

Determination of Fluence Rate for the Monochromatic Radiation Source

The fluence rate was determined using an Oriel 7102 thermopile (Oriel Scientific Ltd, Kingston-upon-Thames, UK). The output voltage was measured on a microvoltmeter (Keithley Instruments, Model 150B) and the microvolt reading converted to $Jm^{-2}s^{-1}$ using a calibration factor for the thermopile. The thermopile was calibrated by chemical actinometry according to the method described by Jagger (1967). The actinometry solution used was potassium ferrioxalate ($K_3Fe(C_2O_4).3H_2O$) which absorbs light completely throughout the UV region. The light catalyzed production of ferrous ion in this solution is the basis of this method of actinometry. Briefly, the number of moles of ferrous ion produced in the actinometry solution during irradiation at a chosen wavelength was determined from a calibration curve of optical density, at 510 nm, against amount of ferrous ion (fig. 17). This value, together with constants for the chosen wavelength (given in

Jagger, 1967) were used to calculate the fluence rate. A number of fluence rates were obtained by altering the distance of the thermopile from the source. Figure 18 shows the relationship of the UV fluence rate obtained by chemical actinometry to the thermopile reading in millivolts. The slope of this line, obtained by linear regression analysis was 0.023, which is the calibration factor for millivolts to $Jm^{-2}sec^{-1}$ for this thermopile.

3) Far-UV Radiation

Source

The source used to provide far-UV (254 nm) radiation was a 5 cm Penray lamp (UV Products Inc, JnC SC-1). This was fitted with a G-275 filter so that 95% of the emitted radiation was 254 nm wavelength. The apparatus is illustrated diagrammatically in fig. 19. The irradiation vessel was positioned 15 cm below the radiation source. A shutter, as described for the monochromatic radiation source, positioned between the source and the irradiation vessel, was used to regulate exposure. The shutter was fitted with a cable release and exposure was timed with a stopclock. Compressed air was blown between the Penray lamp and the upper surface of the shutter to prevent accumulation of ozone. Mains electricity was supplied to the lamp through a voltage stabilizer (Advance Electronics CV100A). The irradiation vessel was a jacketed glass bowl with a 5 cm internal diameter. Ten millilitres of cell suspension was irradiated in the bowl and mixing was achieved by blowing filtered, humidified air through a side opening and over the surface of the suspension.

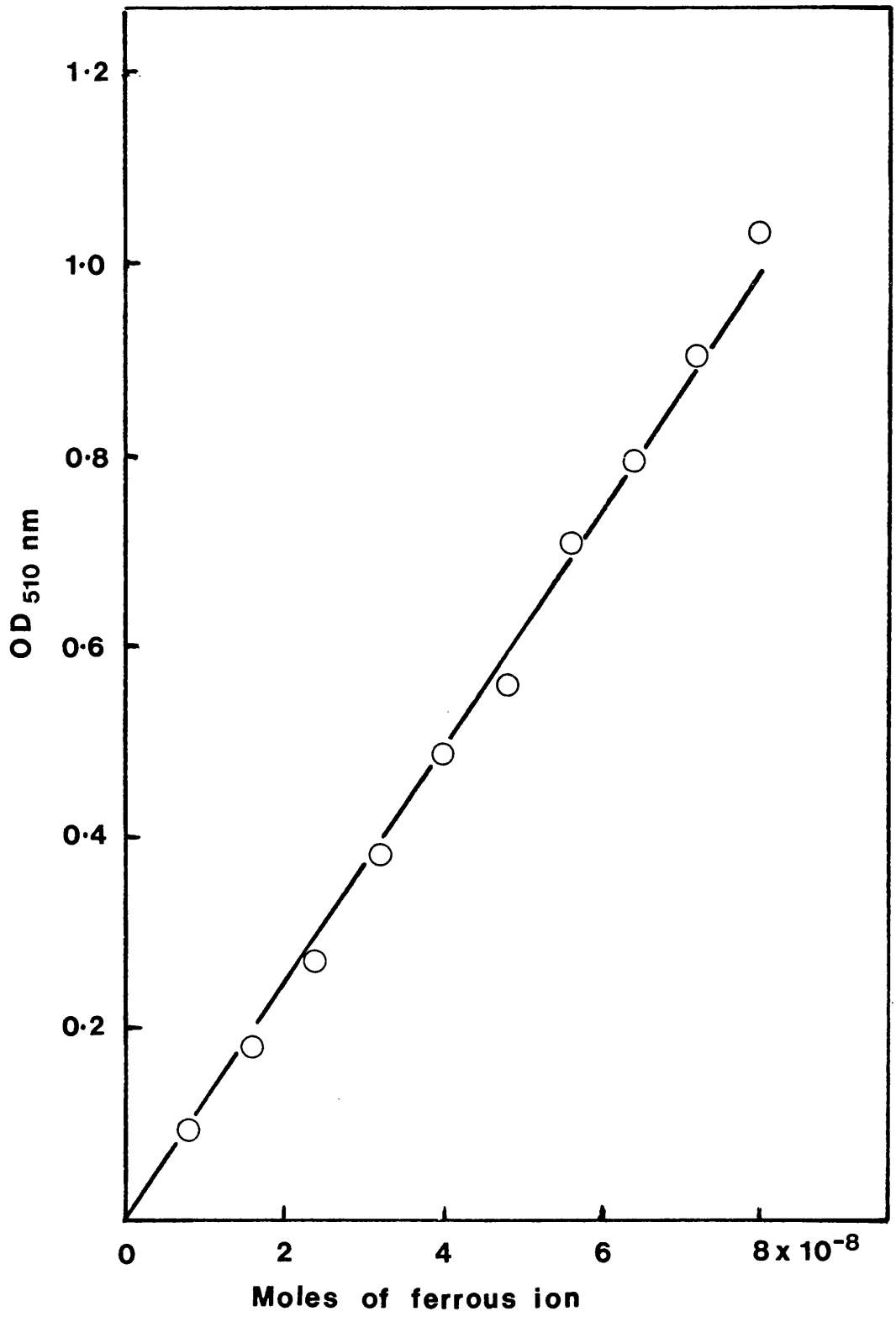


Figure 17: Calibration curve for the conversion of optical density to amount of ferrous ion.

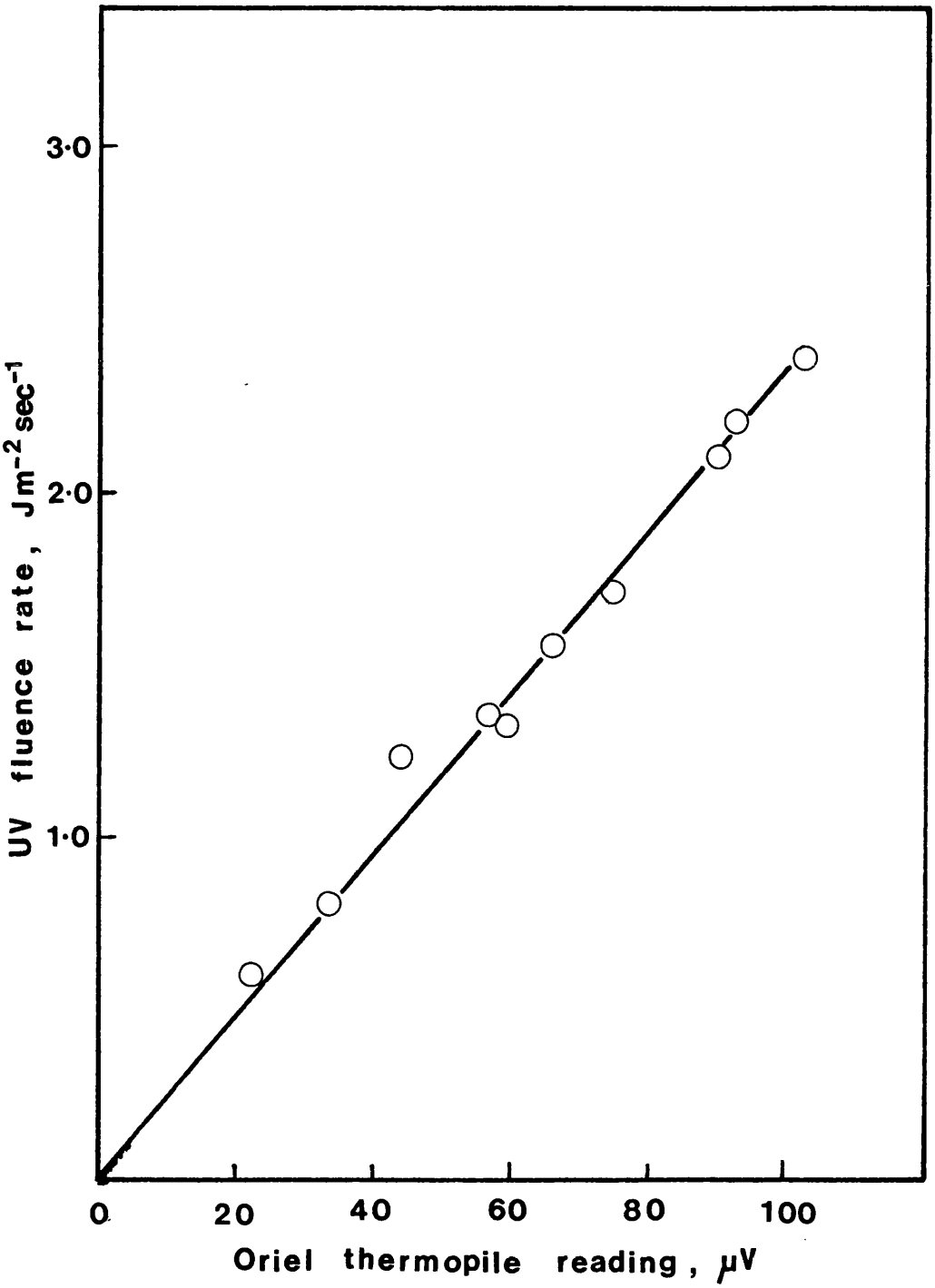


Figure 18: Thermopile reading (Oriel 7102) in microvolts, plotted against UV fluence rate obtained by potassium ferrioxalate actinometry.
Slope by linear regression analysis = 0.023
Correlation coefficient = 0.987.

General Far-UV Irradiation Procedure

The lamp was allowed to "warm-up" for 20 min before use. The fluence was determined by positioning a thermopile (Oriel products, model 7102) below the lamp as shown in fig. 19. The irradiation bowl was removed and the thermopile raised so that it was the same distance from the lamp as the cell suspension would normally be during irradiation. A number of microvolt readings were taken and together with a previously determined calibration figure (see monochromator dosimetry section above) the incident fluence was calculated.

Normally the irradiation bowl was sterilized by dry heat at 160°C for a minimum of 1 hour. Occasionally, when the bowl was to be used in consecutive experiments, it was sterilized by exposure to 254 nm radiation, by opening the shutter for 15 min before use. Irradiation was carried out at room temperature.

The results given in the experimental sections of this study are represented by a single typical experiment. All experiments were repeated two to four times. The data pertaining to each figure is given in Appendix B. Table 1 of Appendix B gives the full data for that particular experiment, further tables give the time of exposure and the surviving fractions only. In addition, the tables presented in Appendix B give data for replicate experiments of those presented in the results sections.

In each of the results sections, additional methods and materials that are directly relevant to that section only, are described.

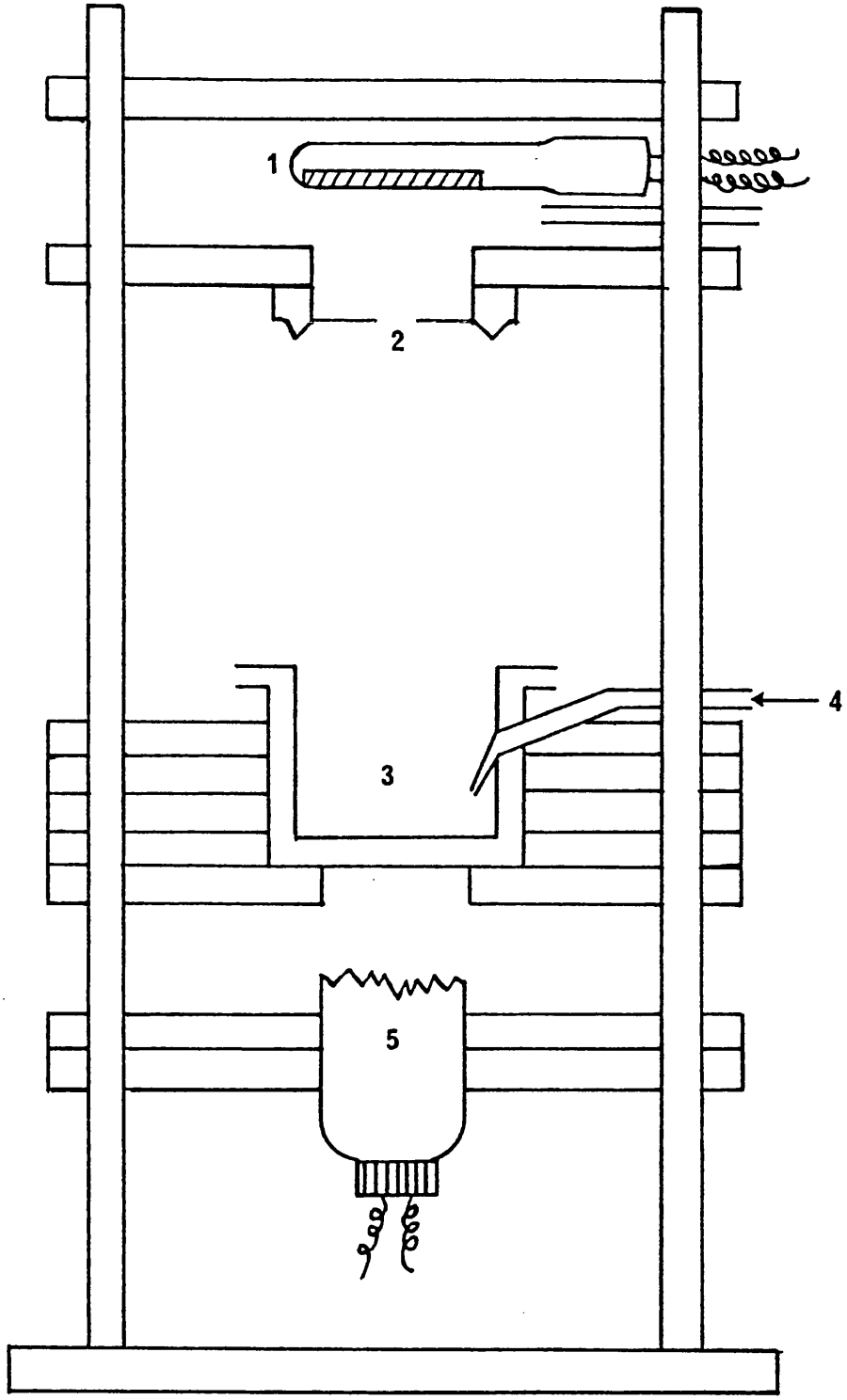


Figure 19: Diagram of apparatus for 254 nm irradiation.

1. UV Source
2. Shutter
3. Irradiation Vessel
4. Air Inlet
5. Position of thermopile

RESULTS: SECTION 1

Effects of peroxide and catalase on near-ultraviolet radiation sensitivity in *Escherichia coli* strains.

It is well established that when cells are irradiated with UV radiation, direct photochemical reactions lead to DNA damage. This damage to DNA is subject to a number of enzymatic repair systems, as discussed in the introduction, some of which, in particular those controlled by the *recA* gene, are inducible. It has been shown that another inducible repair system exists which responds not to direct photochemical damage to DNA, but to so called oxidative damage, in particular that produced by hydrogen peroxide (Demple and Halbrook, 1983). It is also known that hydrogen peroxide is produced by the photo-oxidation of tryptophan by radiation in the 300-400 nm region (McCormick *et al.*, 1976). It has therefore been postulated that oxidative damage and its inducible repair are involved in near-UV radiation effects.

This section of results describes investigations, using a broad-band, near-UV radiation source, which were carried out to examine the roles of hydrogen peroxide and catalase in near-UV radiation induced damage with reference to the inducible repair systems that respond to oxidative damage.

For the group of experiments described in this section, some additional materials and methods were used which were not included in the general methodology section.

Materials

(1) Casamino Acids Solution

This was prepared as a 20% w/v stock solution by dissolving casamino acids (20 g, Difco Laboratories, Detroit, USA) in warm distilled water (70 ml) and, after allowing to cool, making up to 100 ml with cold distilled water. The solution was filter sterilized using a 0.2 µm pore size membrane filter contained in a sterile Swinnex unit (Millipore UK Ltd,

London). This stock solution was stored at 4°C and was diluted 100-fold to give a final concentration of 2 mg/ml.

(2) Hydrogen Peroxide

Hydrogen peroxide (H_2O_2; Fluka AG) was diluted to three per cent with distilled water and filter sterilized as described above. Fresh stock solutions were prepared weekly and stored at 4°C. Before use, the stock solution was diluted as required in sterile M9 salts solution.

(3) Catalase and Catalase Plates

A 0.1 mg/ml solution of catalase was prepared as required by diluting catalase (0.1 ml; Boehringer Mannheim; 20 mg/ml catalase suspension from beef liver; 65000 units/mg) in 19.9 ml M9 salts solution. After the catalase suspension had dissolved, the solution was filter sterilized using a 0.45 µm pore size membrane filter. The solution was used to assess the effect of catalase on survival by spreading 0.2 ml of the catalase stock solution over the surface of an overdried tryptone agar plate, using a sterile glass spreader, to give a final concentration of 1 µg/ml. Once the catalase solution had been absorbed by the plating medium, the plates were ready for use.

(4) Superoxide Dismutase (SOD) and SOD Plates

A 1 mg/ml solution of SOD was prepared by dissolving SOD (10 mg: Boehringer Mannheim; bovine SOD 5000 units/mg) in 10 ml M9 salts solution. The solution was sterilized by filtration using a 0.2 µm pore size membrane filter. The solution was diluted to the required concentration in M9 salts solution and 0.2 ml aliquots spread over the surface of agar plates as described above for catalase.

(5) Titanium Oxysulphate

A stock solution of 28 mM titanium oxysulphate ($TiOSO_4$, Sun Valley Research; kindly donated by K. C. Smith, Stanford, California, USA) was made by dissolving 4.48 g $TiOSO_4$ in 800 ml 2N sulphuric acid and making up to 1 l in a volumetric flask. The solution was stored at room temperature.

Methods

(1) Hydrogen Peroxide Treatment of Bacteria

Exponential phase bacteria were pretreated with H_2O_2 using a method based on that of Tyrrell (1985). Exponential phase cells were obtained by inoculating 100 ml prewarmed growth media with 1 ml stationary (24 h) phase cells. The cells were grown at 37°C, aerated and shaking in a water bath (as described in the general methodology) for precisely 75 min. At this point, two 20 ml samples were transferred to clean, sterile, prewarmed 250 ml culture vessels. One was pretreated with 30 µM H_2O_2 and the other used as an unpretreated control. The cultures were grown for a further 60 min (giving a total growth time of 135 min) before harvesting (as described in the general methodology). The resultant cell suspensions were diluted in M9 salts solution to give final cell counts of 10^7 CFU/ml. Five millilitre samples of the cell suspensions were either irradiated as described previously or treated with 5 mM H_2O_2.

For H_2O_2 treatment, 4.75 ml of each cell suspension was transferred to a sterile 100 ml beaker, capped with aluminium foil, and placed in a shaking water bath at 37°C. The cell suspensions were allowed to warm up for 5 min before adding 0.25 ml 100 mM H_2O_2, giving a final concentration of 5 mM H_2O_2. A 0.2 ml sample of each cell suspension was taken immediately before addition of 5 mM H_2O_2, as a zero control, diluted in M9

and plated as previously described. Subsequent exposure to 5 mM H_2O_2 was timed with a stopclock. The addition of 5 mM H_2O_2 to the various cell suspensions was staggered to allow time for sampling and serial dilution.

(2) Assay for Peroxide Scavenging Ability

Peroxide scavenging ability of exponential phase cells was determined using a method similar to that of Sun *et al.* (1975). The assay utilizes the formation of a yellow coloured complex between the reagent, $TiOSO_4$, and available H_2O_2. Exponential phase cells were pretreated with 30 µM H_2O_2 as described above and harvested by membrane filtration. The cell suspensions were diluted in M9 salts solution to give a cell concentration of $2x10^8$ CFU/ml. Five millilitres of each cell suspension were transferred to a sterile 100 ml beaker capped with aluminium foil, and allowed to equilibriate to room temperature for 5 min. The cells were then exposed to 5 mM H_2O_2 by the addition of 5 ml 10 mM H_2O_2. A 0.5 ml sample was immediately taken and added to 2 ml $TiOSO_4$ in a clean, glass test-tube. Samples were taken over a period of time and exposure timed with a stopclock. The optical density of the resultant yellow reaction product was read on a spectrophotometer (Unicam Instruments, SP600) at 405 nm. The optical density was read within 30 min of sampling since the yellow reaction product tended to fall out of solution when left standing. Samples were read against a blank consisting a 1 ml cell suspension, 1 ml distilled water and 4 ml $TiOSO_4$. The assay measures the loss of H_2O_2 and thereby, indirectly the H_2O_2 scavenging ability of the cells. Results were plotted as change in optical density, as compared to the zero time reading, against the length of exposure to 5 mM H_2O_2, in minutes. Thus, an increase in change of optical density corresponds to an increase in the H_2O_2 scavenging ability of the cells.

Results

The experimental results described in this section were all obtained with exponential phase cells, grown for 135 min in defined growth media. The defined media used is described in the general methodology, with the exception of that for *E. coli* K12 AB1157 where the individual amino acid solutions were replaced with the single casamino acids solution. The cells were irradiated or treated with H_2O_2 as a $1x10^7$ CFU/ml suspension in M9 salts solution. Cells were irradiated in the central positions (positions 5, 6, 7 and 8) of the black-light blue lamp-box, in which positions it had previously been shown that the fluence rates were not significantly different (fig. 14, general methodology). The plating media used was tryptone agar throughout.

Sensitivity of Strains to 5 mM Hydrogen Peroxide

An initial series of experiments was carried out on five bacterial strains to examine whether a protective response to H_2O_2 could be induced by pretreating cells with a low, non-lethal dose of H_2O_2. The five strains used in this section of results were *E. coli* K12 AB1157, *E. coli* B/r and *E. coli* SR385 (a K12 derivative), three repair competent strains that are widely used in this department and other laboratories, the catalase deficient mutant *E. coli* UMI and its isogenic, repair competent parent strain, *E. coli* CSH7.

Exponentially growing cells were pretreated with 30 µM H_2O_2 for 60 min at 37°C. After harvesting, the cells were exposed to 5 mM H_2O_2 in M9 salts solution at 37°C for upto 80 min. The resultant survival curve for *E. coli* K12 AB1157 is shown in fig. 20 with the corresponding data presented in Appendix B, Table 1.

Table 1 (Appendix B) presents the data pertaining to fig. 20 in full in order to make some important points. The viable count per millilitre is calculated from the dilution and the mean plate count. The surviving fraction is the ratio of surviving cells (i.e. viable count) after treatment, to the initial number of viable cells. It is important that the initial viable count is the same in the different samples within an experiment since some degradation of H_2O_2 is to be expected by the cells themselves. Widely different initial viable counts could lead to variations in the effective H_2O_2 dose received. The need for similar cell densities between samples irradiated together in one experiment is also important, as variations in the optical densities of the cell suspension can lead to screening of cells from the near-UV radiation.

Having made these points for the data shown in Table 1, further tables pertaining to experimental data (presented in Appendix B) will show only the H_2O_2 challenge or radiation fluence (in minutes) and the corresponding surviving fractions. The data from one replicate experiment is also shown for each table except where results were ambiguous. In these cases, further replicate experiments are presented.

Figure 20 shows that without 30 µM H_2O_2 pretreatment, *E. coli* K12 AB1157 has an initial shoulder region followed by a rapid increase in sensitivity to 5 mM H_2O_2. Cells that had been pretreated with 30 µM H_2O_2 exhibited a gradual decrease in survival over the same period of exposure with no rapid increase in sensitivity. The same experiment was carried out with *E. coli* B/r and *E. coli* SR385 and the survival curves for these two experiments are shown in fig. 21. The response of *E. coli* B/r (fig. 21A) to 5 mM H_2O_2 challenge without 30 µM H_2O_2 pretreatment was similar to that of *E. coli* K12 AB1157 (fig. 20) with less than 1 log kill after 80 min exposure to 5 mM H_2O_2.

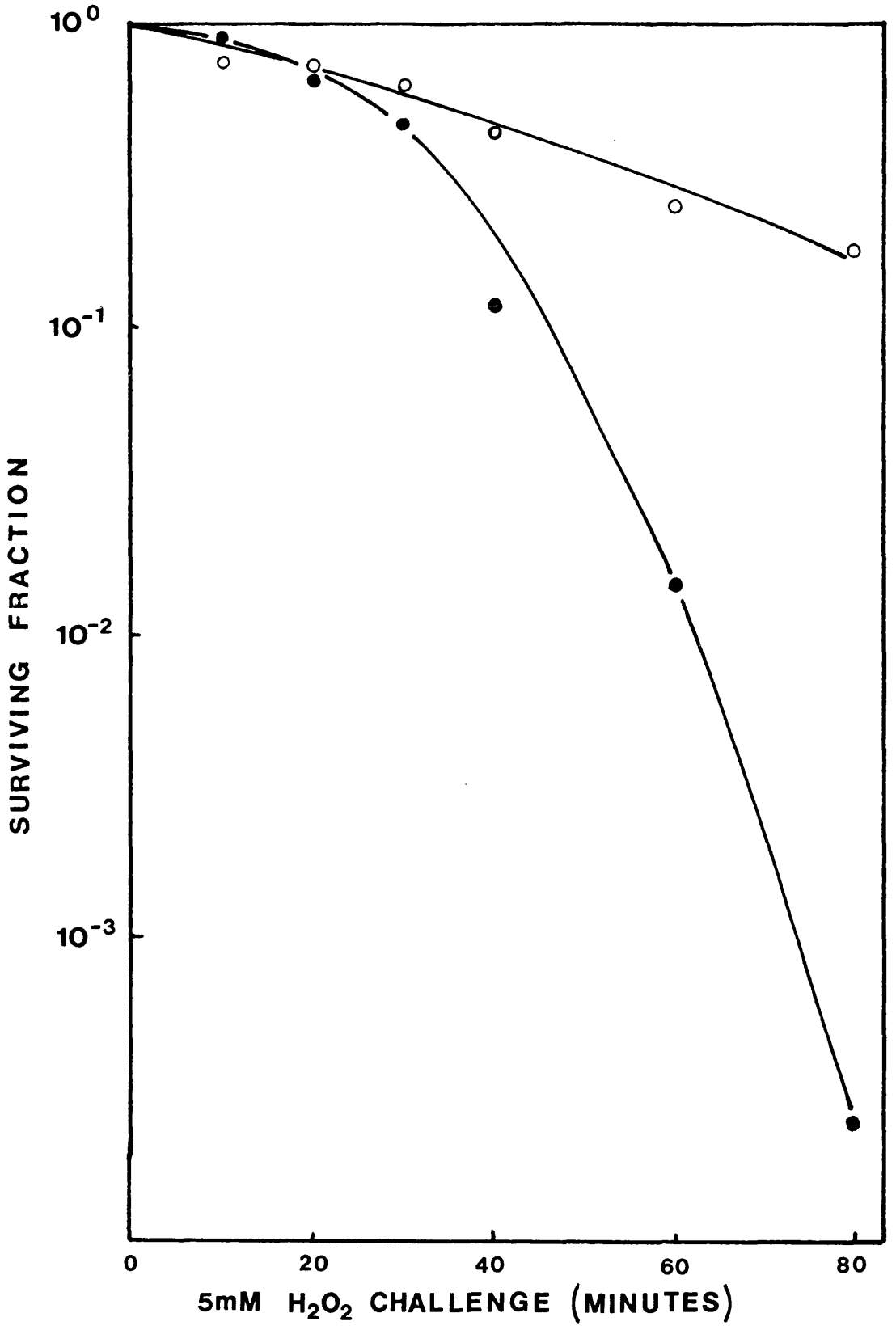


Figure 20: Survival curves for *E. coli* K12 AB1157 after 5 mM H_2O_2 challenge with 30 µM H_2O_2 pretreatment (○) and without pretreatment (●).

Results obtained with *E. coli* SR385 are shown in fig. 21B. The response of unpretreated cells was similar to that seen with both B/r and AB1157 (figs. 20 and 21A). However, the increased resistance to 5 mM H_2O_2 produced by pretreating cells with 30 µM H_2O_2 seen with both AB1157 and B/r was less marked with SR385. Although there was an initial increase in the resistance to 5 mM H_2O_2 as compared to that seen with the unpretreated cells, this was followed by a rapid increase in sensitivity which tailed off at higher doses.

The induction of bacterial resistance to H_2O_2 has been previously reported. Both Demple and Halbrook (1983) and Tyrrell (1985) used *E. coli* K12 AB1157 to assess the affect of 30 µM H_2O_2 pretreatment on the subsequent survival of cells exposed to 5 mM H_2O_2. Both studies showed that pre-exposure to low concetrations of H_2O_2 enhanced resistance to a lethal H_2O_2 challenge in growing cells. Both studies also showed that growing cells challenged with 5 mM H_2O_2 showed an initial rapid increase in sensitivity which decreased with time. Cells pretreated with 30 µM H_2O_2 increased in resistant to 5 mM H_2O_2 and continued to do so throughout the challenge period.

The differences seen between these published results and those presented in fig. 20 may be explained by differences in experimental procedure. Firstly, the results described in this study were obtained by challenging cells held in buffer with 5 mM H_2O_2 whereas the results of Demple and Halbrook (1983) and Tyrrell (1985) were obtained by challenging cells growing in full media with 5 mM H_2O_2. The induction response of growing cells will be far more complex than that of cells held in buffer since, with the former method, the H_2O_2 concentration will be decreased with time by growing survivors and H_2O_2 may also be quenched by components of the growth medium. However, Tyrrell (1985) also presented results for

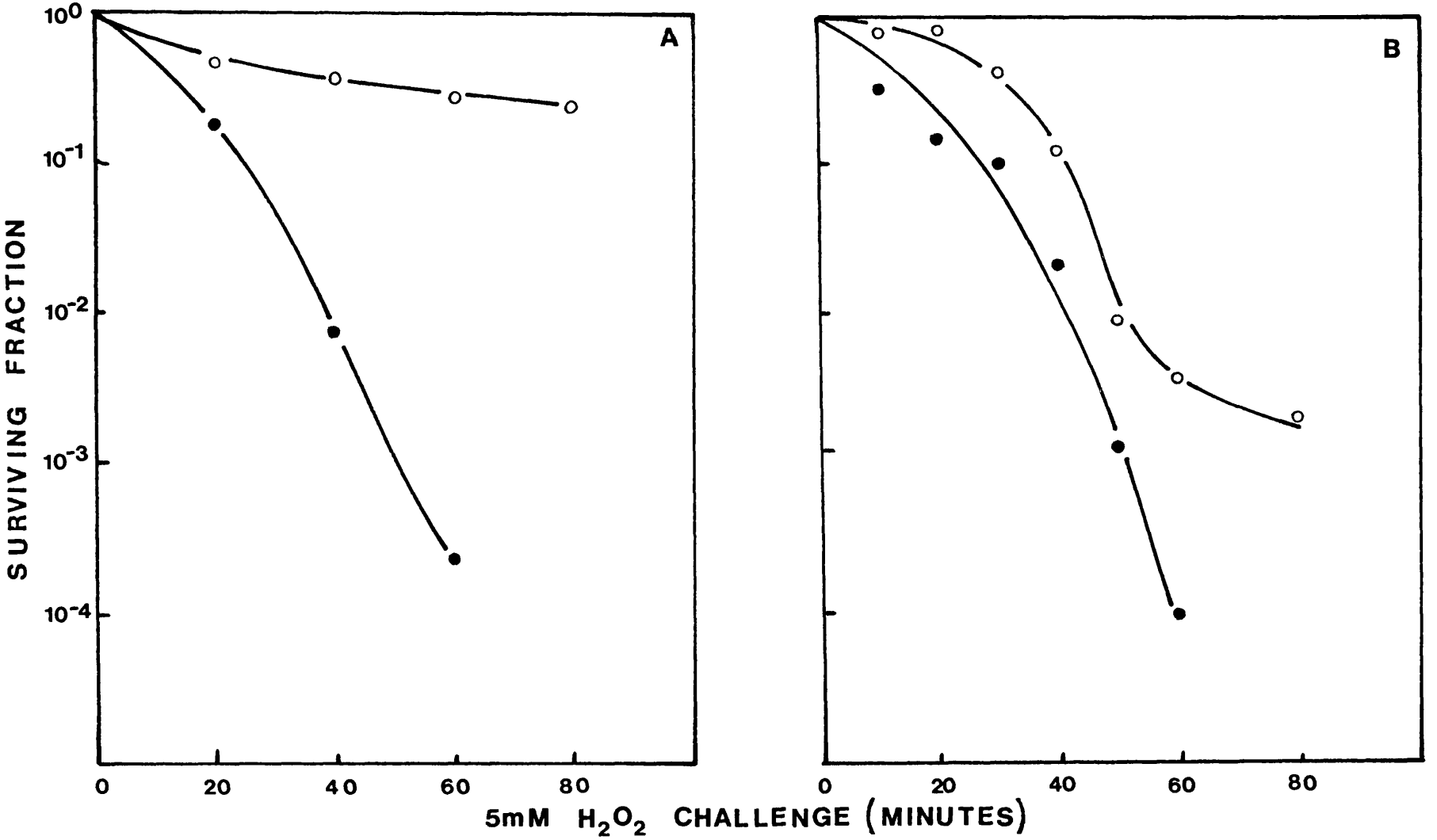


Figure 21: Survival curves for (A) *E. coli* B/r and (B) *E. coli* SR385 after 5 mM H_2O_2 challenge with 30 µM H_2O_2 pretreatment (o) and without pretreatment (●).

E. coli K12 AB1157 challenged with 5 mM H_2O_2 in buffer and these results agree well with those presented in fig. 20. Secondly, Demple and Halbrook (1983) pretreated cells with 30 µM H_2O_2 for 30 min rather than the 60 min pre-exposure time used in this study. Tyrrell (1985) found that although 30 min pretreatment did induce resistance to subsequent H_2O_2 challenge, the effect was not maximal. A maximum response was seen with 60 min pre-exposure time with the effect decreasing with longer pretreatment exposures.

Demple and Halbrook (1983) suggest that pretreatment of *E. coli* cells with sublethal concentrations of H_2O_2 results in resistance to oxidative damage from lethal concentrations of H_2O_2 because a repair system specific to oxidative damage is induced. They report that this induction acts at the level of DNA repair since the ability of cells to reactivate (repair) the damaged DNA of oxidized bacteriophage is increased in pretreated host cells. However, other workers have failed to reproduce this result and have suggested that the protective system induced by pretreatment of cells with sublethal H_2O_2 concentrations may increase levels of protective enzymes that degrade H_2O_2 (Sammartano and Tuveson, 1985). One such enzyme, catalase, which breaks down H_2O_2, is known to be inducible (Richter and Loewen, 1981) and Demple and Halbrook (1983) have shown that H_2O_2 pretreatment increased catalase activity 2 to 4 fold. However, this was variable and correlated poorly with the degree of resistance induced in the bacteria.

If catalase was the product of the inducible response described above, it would be expected that cells lacking endogenous catalase would be unable to induce this protective response. To examine this possibility, *E. coli* CSH7 and its derivative *E. coli* UMI were pretreated with 30 µM H_2O_2 and

challenged with 5 mM H_2O_2. The resultant survival curves are shown in figs. 22A and B.

Escherichia coli UMI is a derivative of CSH7 (Loewen, 1984) which carries the mutations *katE1* and *katG14* affecting the synthesis of the monofunctional catalase HPII and the bifunctional isoenzyme pair HP1-A and -B that possess both catalase and peroxidase activity. As such, UMI might be expected to be highly sensitive to H_2O_2. The mutant UMI was indeed found to be more sensitive to 5 mM H_2O_2 than AB1157, B/r, SR385 and CSH7 although by a smaller margin than might have been expected (fig. 22A). Pretreatment with 30 µM H_2O_2 did not induce a protective response in UMI, the induction process causing an increase rather than a decrease in sensitivity. Controls carried out with *E. coli* CSH7, the parent strain of UMI, exhibited protection by pretreatment only after approximately 30 min 5 mM H_2O_2 challenge (fig. 22B). However, with CSH7, this response was variable, although some degree of protection by pretreatment was always seen. The results obtained with UMI imply that pretreatment with H_2O_2 involves the induction of catalase which helps protect the cells from subsequent H_2O_2 challenge.

Sensitivity to Broad-Band Near-UV Radiation

Hydrogen peroxide is known to be a toxic photoproduct produced by the near-UV irradiation of tryptophan (McCormick *et al.*, 1976). Since peroxides give rise to damaging oxygen species, the production of H_2O_2 during near-UV irradiation could lead to the production of reactive species and subsequent damage. Active oxygen species have already been implicated in near-UV radiation induced damage (Webb, 1977). It would therefore appear that both near-UV radiation and H_2O_2 inactivate cells by forming oxygen intermediates which damage DNA. Thus is would seem reasonable to

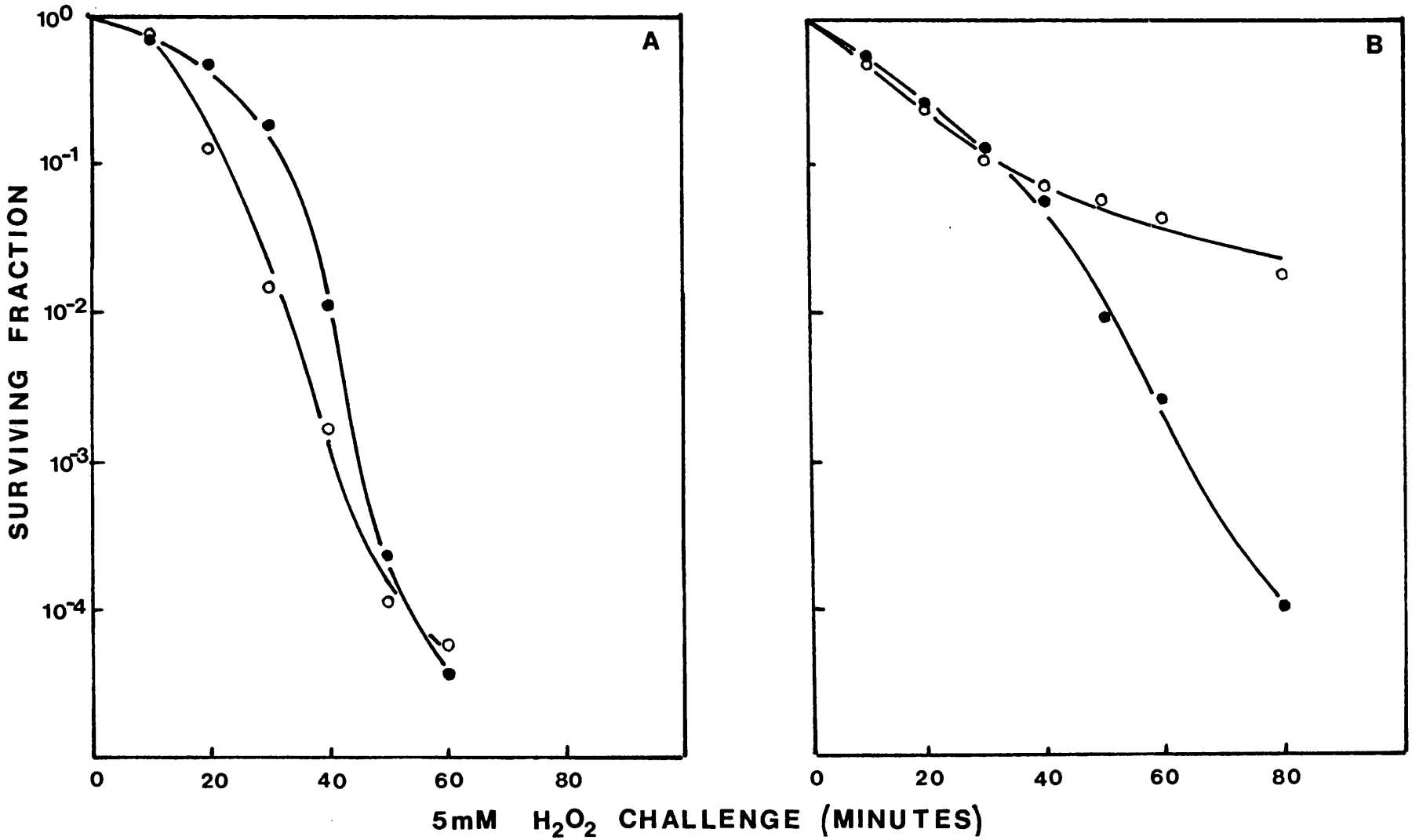


Figure 22: Survival curves for (A) *E. coli* UMI and (B) *E. coli* CSH7 after 5 mM H_2O_2 challenge with 30 µM H_2O_2 pretreatment (o) and without pretreatment (●).

expect that pretreating cells in such a way as to induce protection against H_2O_2 would also protect them from subsequent near-UV radiation damage.

In this subsection of results, the five strains used above were pretreated with 30 μM H_2O_2 and then, instead of the H_2O_2 challenge, were exposed to broad-band near-UV radiation.

In contrast to their similar responses to H_2O_2, exponential phase K12 AB1157 and B/r exhibited very different responses when exposed to broad-band near-UV radiation. Without pretreatment, K12 AB1157 (fig. 23A) showed an initial shoulder region extending over approximately 60 min before rapidly increasing in sensitivity. The corresponding survival curve for B/r (fig. 23B) showed an initial high sensitivity that decreased at higher fluences. After pretreating cells with 30 μM H_2O_2, K12 AB1157 exhibited a small, though reproducible decrease in sensitivity (fig. 23A). However, the effect seen with B/r (fig. 23B) was very marked, with the survival of pretreated cells exceeding that of pretreated K12 AB1157.

The survival curves obtained for the same experiment using *E. coli* SR385 are shown in fig. 24. The results obtained are similar to those seen with K12 AB1157, with the H_2O_2 pretreatment inducing a small though reproducible increase in resistance to near-UV radiation.

The enhanced protection to H_2O_2 and near-UV radiation produced by pretreatment with low, non-lethal levels of H_2O_2, seen in the above three strains, is in agreement with work published by Tyrrell (1985). He observed that pretreatement with both near-UV radiation and H_2O_2 protected K12 AB1157 against inactivation by a lethal dose of the other agent. The common induction process observed by Tyrrell (1985) and seen in figs. 20 to 24 does suggest that there is a common pathway for protection of damage by H_2O_2 and near-UV radiation. However, other factors may also be involved

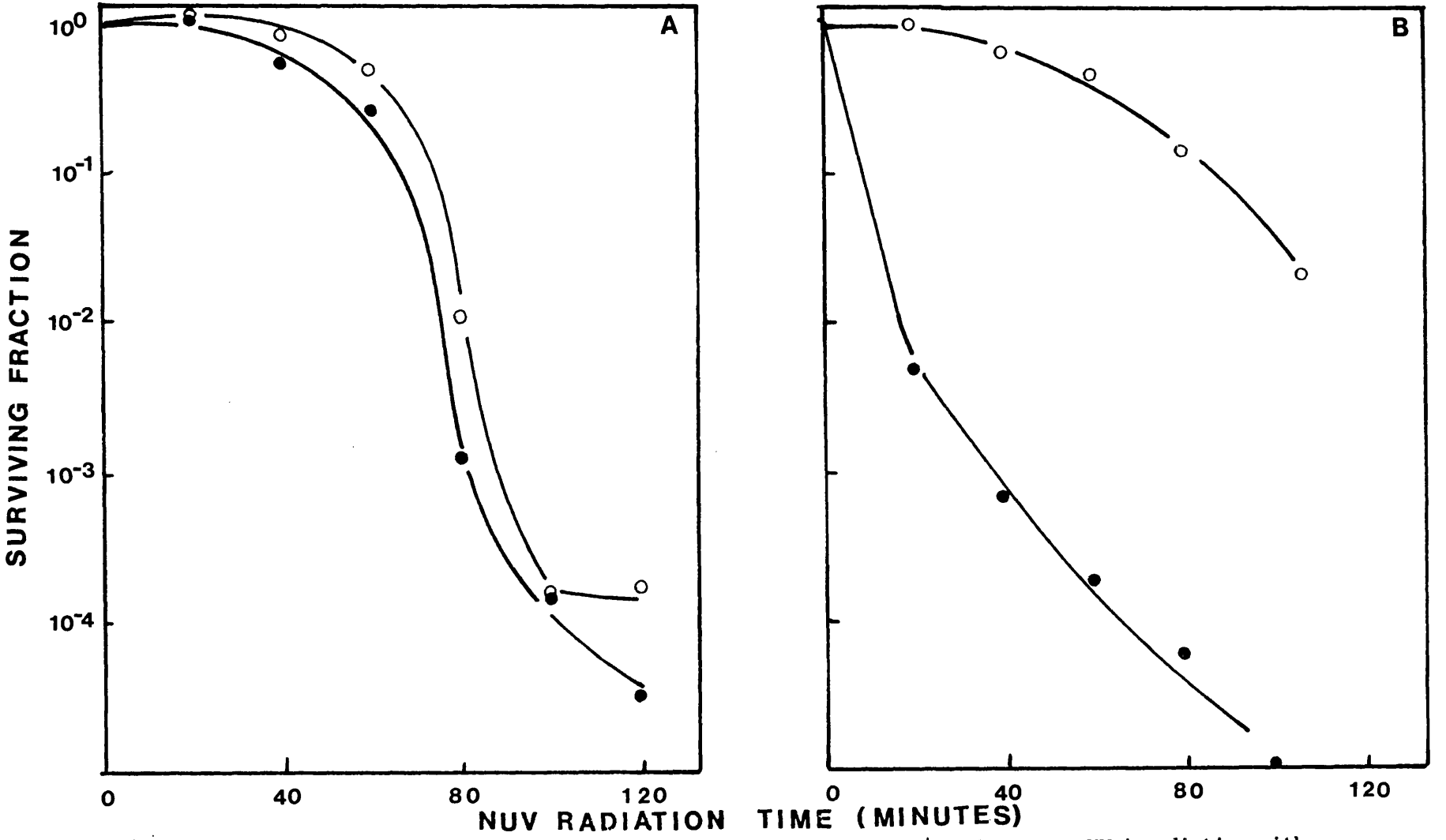


Figure 23: Survival curves for (A) *E. coli* K12 AB1157 and (B) *E. coli* B/r after near-UV irradiation with 30 μM H_2O_2 pretreatment (○) and without pretreatment (●).

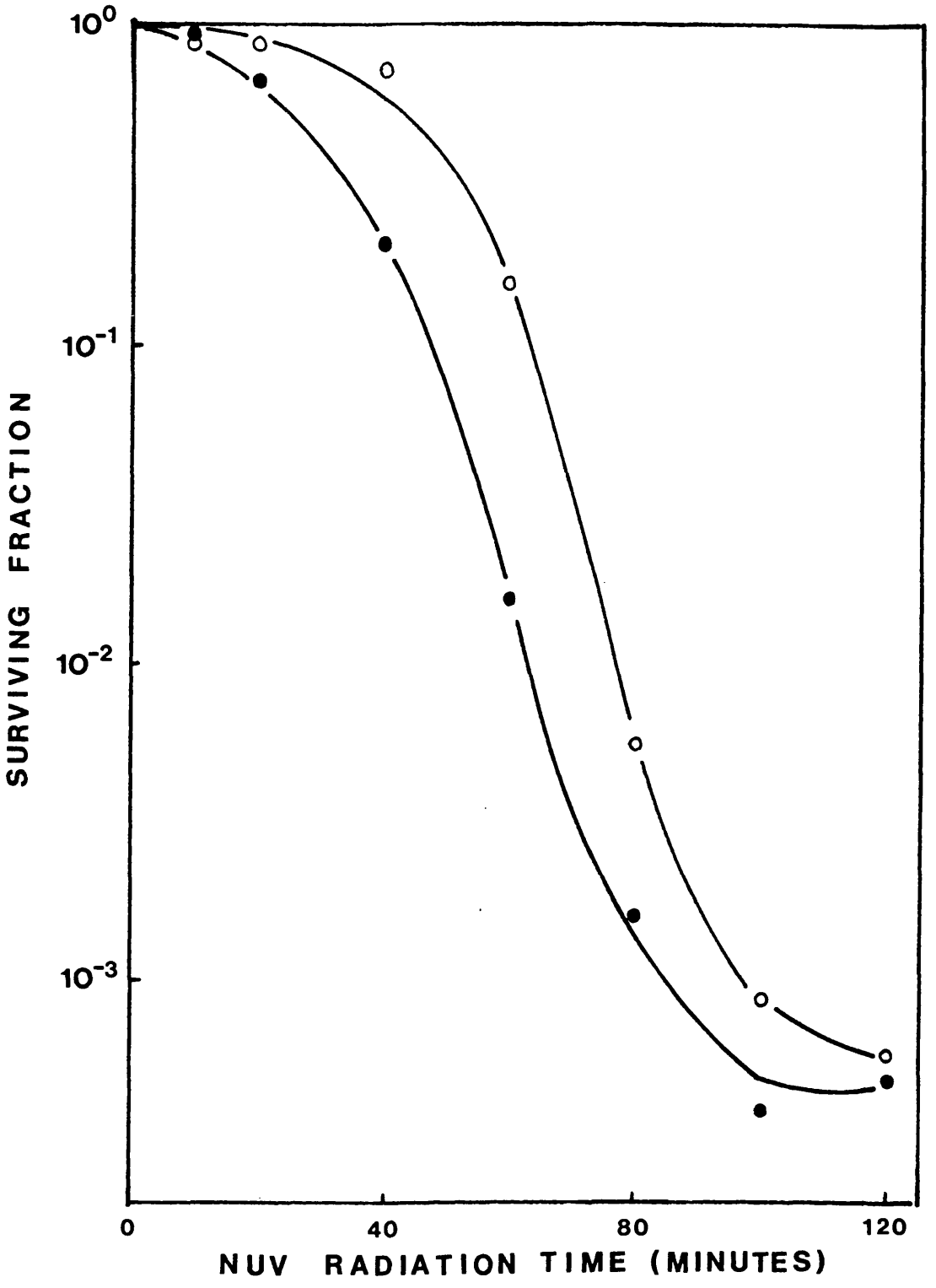


Figure 24: Survival curves for *E. coli* SR385 after near-UV irradiation with 30 μM H_2O_2 pretreatment (o) and without pretreatment (●).

since the high sensitivity of exponential phase B/r to near-UV radiation is not entirely consistent with the proposal that H_2O_2 has a major role in near-UV radiation damage. Although K12 AB1157, B/r and SR385 have similar responses to 5 mM H_2O_2 challenge, they have widely differing responses to near-UV radiation. If their responses to near-UV radiation were directly and solely related to H_2O_2 produced during irradiation, it would be expected that they would have similar responses after challenge with either near-UV radiation or H_2O_2.

The role of H_2O_2 in near-UV radiation damage was further examined by performing the H_2O_2 pretreatment and near-UV radiation challenge experiment on the catalase deficient mutant *E. coli* UMI. The result of this experiment is shown in fig. 25A. As was seen with the 5 mM H_2O_2 challenge experiment (fig. 22A), there was no induced protection after 30 μM H_2O_2 pretreatment. Surprisingly, unpretreated UMI showed a similar near-UV radiation sensitivity to that shown by both K12 AB1157 and SR385 and was considerably more resistant than B/r to near-UV radiation. UMI also appeared to be more resistant to near-UV radiation than its parent strain, CSH7 (fig. 25B). CSH7 did, however, show an inducible response.

The results obtained after near-UV radiation treatment of UMI are again inconsistent with the idea of the involvement of H_2O_2 in near-UV radiation damage. The mutant UMI, which should have no catalase either induced or consitutive, would, as such, be expected to be sensitive to H_2O_2 and dependent on the role of H_2O_2 in near-UV radiation inactivation. However, this does not appear to be the case. UMI shows greatest sensitivity to 5 mM H_2O_2 (fig. 22A) of the five strains tested yet is not correspondingly sensitive to near-UV radiation (fig. 25A). Thus the importance of catalase in the protection of unpretreated cells from the lethal effects of near-UV radiation must be questioned. The role of

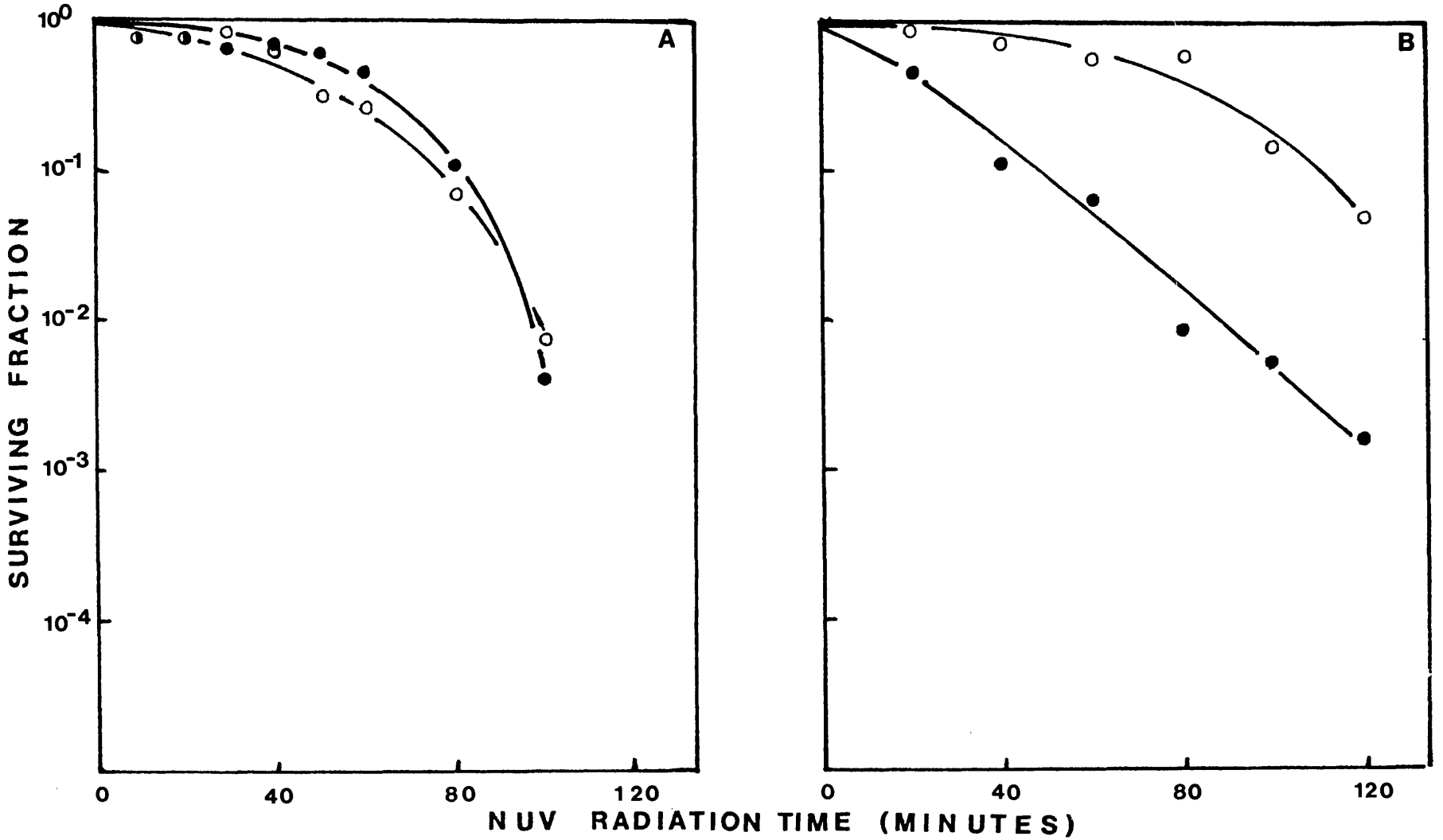


Figure 25: Survival curves for (A) *E. coli* UMI and (B) *E. coli* CSH7 after near-UV irradiation with 30 µM H_2O_2 pretreatment (O) and without pretreatment (●).

catalase in near-UV radiation protection cannot, however, be totally dismissed since the lack of inducible response in the catalase deficient mutant would seem to imply that catalase is involved in the additional protective effect cause by the induction process in the other strains tested.

The Effect of Addition of Catalase to the Plating Media

To try and clarify these apparently conflicting results, the effect of the addition of exogenous catalase was investigated. It has been reported that the addition of catalase to the post-treatment plating medium reduces near-UV radiation sensitivity in *E. coli* strains (Sammartano and Tuveson, 1984). They also found that catalase added to the plating medium after near-UV radiation exposure afforded greater protection than catalase added to the cell suspension during inactivation. This would suggest that if H_2O_2 produced during near-UV radiation exposure decays to give reactive oxygen species, then it does so by a gradual process.

Figures 26, 27 and 29 show the results of duplicate experiments to those represented in figs. 23, 24 and 25. In addition, the cells have been plated out on tryptone agar in the presence of catalase with and without the corresponding pretreatment. In all three figures, added catalase was seen to reduce the near-UV radiation sensitivity as compared to the unpretreated cells that were plated without catalase. In the case of *E. coli* K12 AB1157 (fig. 26A) there is a small protective effect but the magnitude is not sufficient to determine whether or not this is additive in relation to the protection provided by pretreatment with H_2O_2. In the case of *E. coli* B/r (fig. 26B) the addition of catalase provided protection of the same order of magnitude as that provided by pretreatment with H_2O_2. With *E. coli* SR385 (fig. 27) the addition of catalase to the plating medium

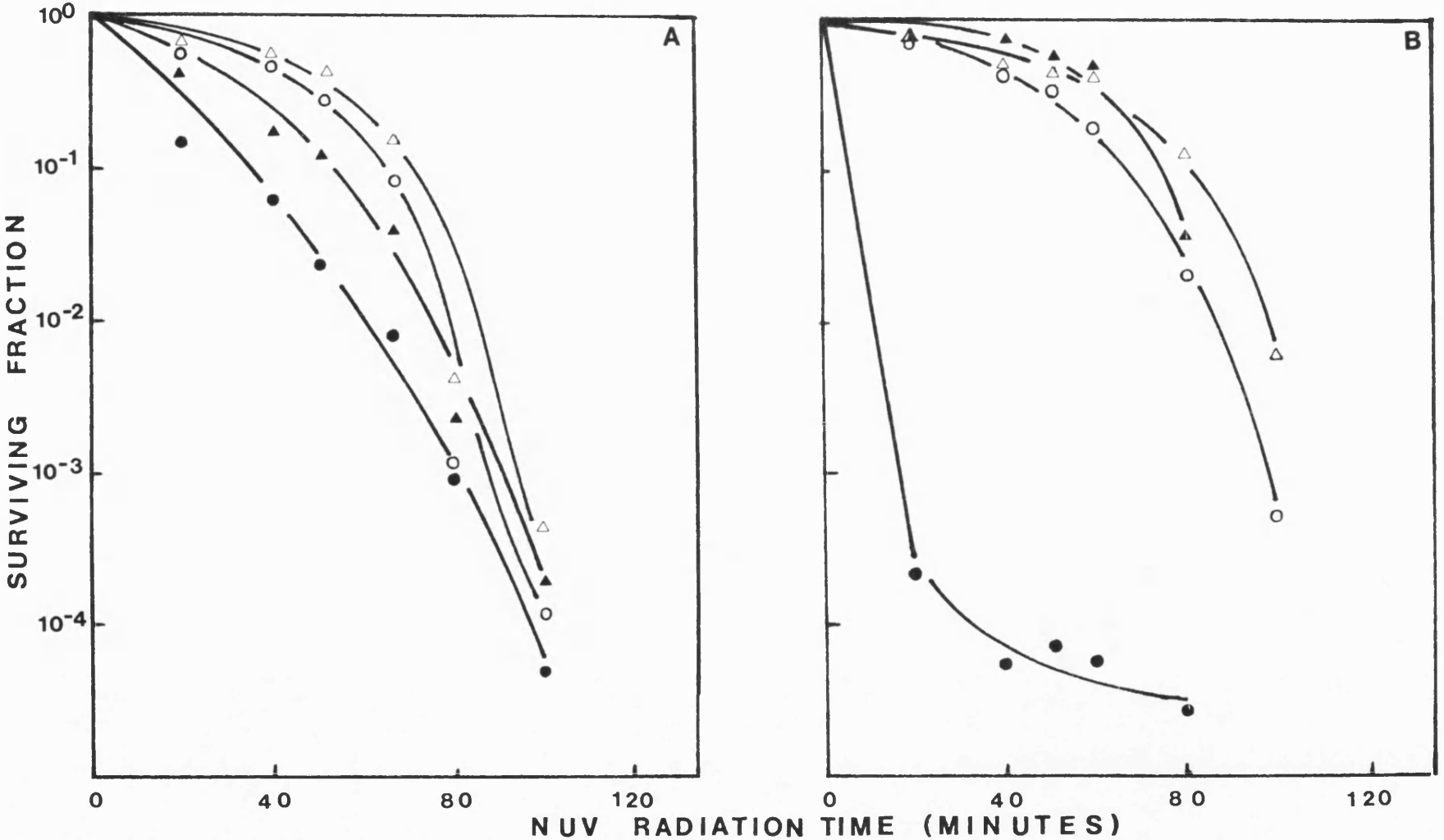


Figure 26: Survival curves for (A) *E. coli* K12 AB1157 and (B) *E. coli* B/r after near-UV irradiation with 30 µM H_2O_2 pretreatment (○, △) and without pretreatment (●, ▲), with catalase in the plating medium (△, ▲) and without (○, ●).

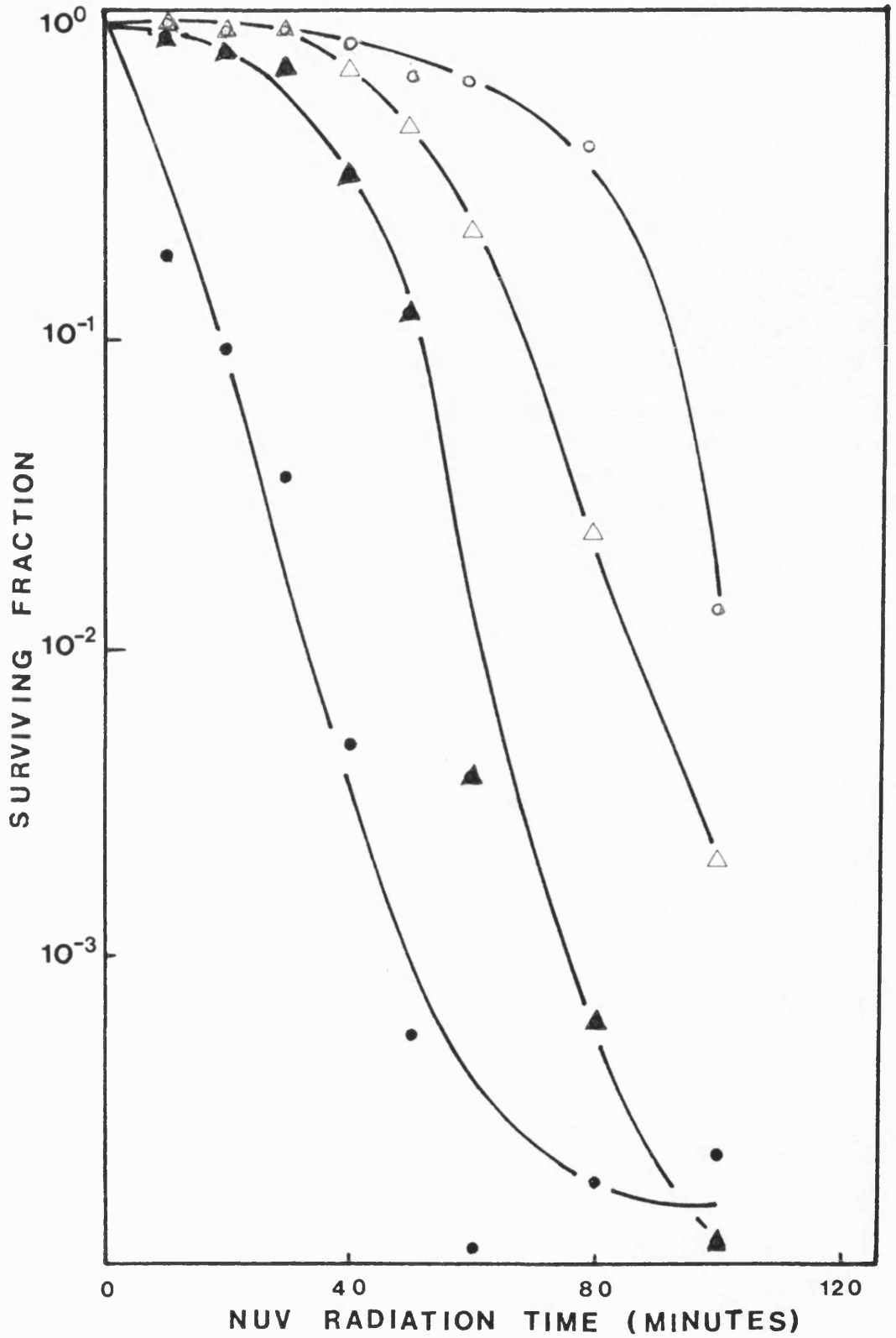


Figure 27: Survival curves for *E. coli* SR385 after near-UV irradiation with pretreatment (O,△) and without pretreatment (●,▲) with the addition of catalase in the plating medium (△,▲) and without catalase (O , ●).

of both pretreated and unpretreated gave an intermediate protective response, although neither reached the level of protection produced by pretreatment alone. In all three cases, the combination of pretreatment and catalase did not provide any marked additional protection over the effects of pretreatment alone or catalase alone.

In an additional experiment, irradiated K12 AB1157 was assessed for viability on tryptone agar containig the enzyme superoxide dismutase (SOD) (fig. 28). In contrast to the results obtained after the addition of catalase to the post-irradiation medium, no marked protection was afforded by SOD at the concentrations tested (10 µg/ml and 100 µg/ml), although at higher fluences a slight increase in survival was seen which increased with increasing SOD concentration.

The effect of the addition of catalase to the post-irradiation plating medium was also exmained in the catalase deficient mutant UMI and its parent strain CSH7. The results of these experiments are shown in fig. 29A and B. In fig. 29A the survival curves for UMI show that, as before, pretreatment does not induce any protective effect, but rather causes some additional sensitization to near-UV radiation. The addition of catalase to the plating medium increased the survival of both pretreated and unpretreated cells. Likewise the addition of catalase to the plating medium of unpretreated cells of *E. coli* CSH7 (fig. 29B) increased survival.

The results obtained in figs. 26-29 confirm the findings of Sammartano and Tuveson (1984) that showed that catalase added to the plating media for four *E. coli* strains protected against inactivation by broad-spectrum near-UV radiation. This response was seen in strains carrying both the *uvrA* and *nur* genes either singly or in combination. The

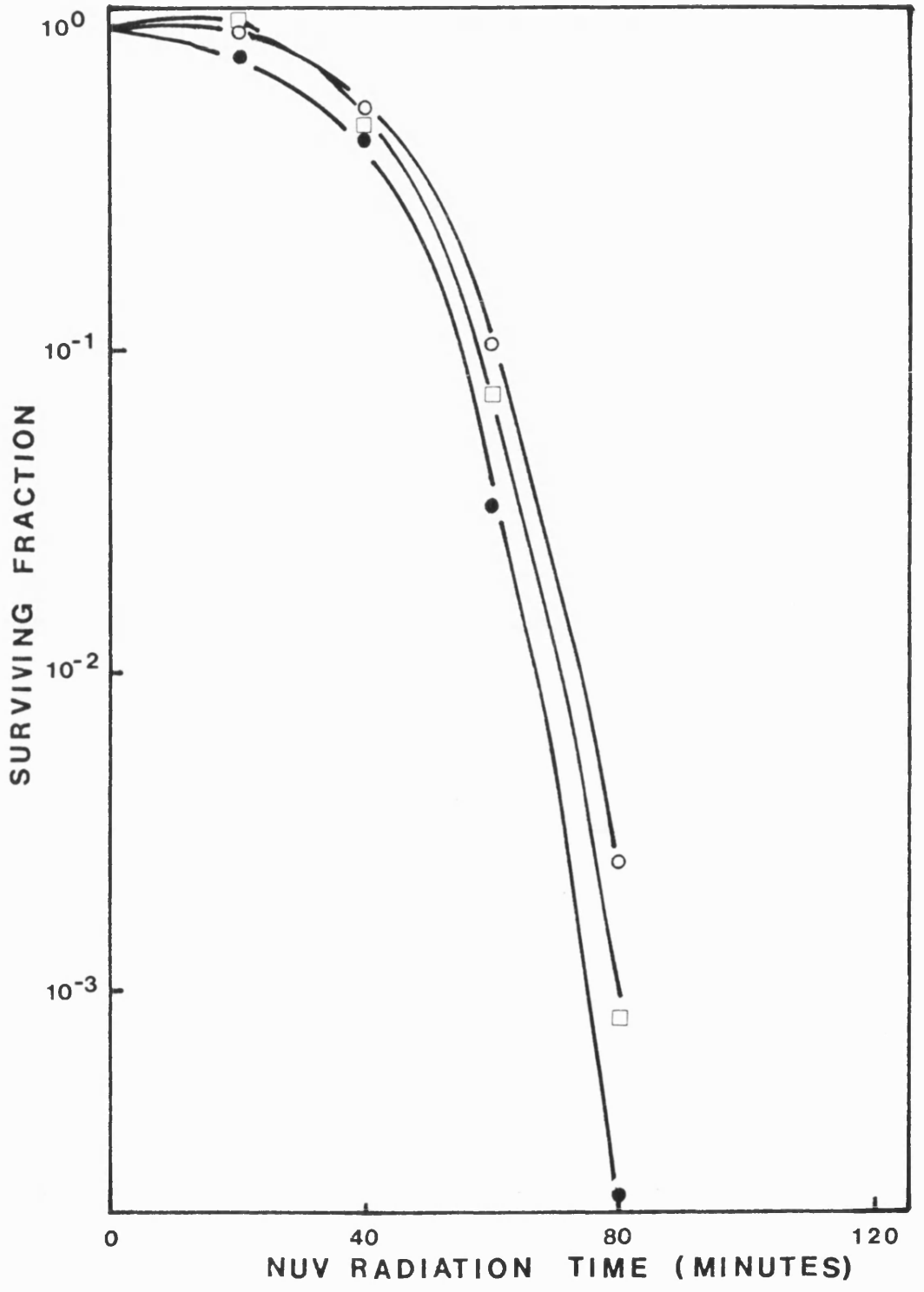


Figure 28: Survival curves for *E. coli* K12 AB1157 after near-UV irradiation without SOD in the plating medium (●) or with SOD present at 10 µg/ml (□) or 100 µg/ml (○).

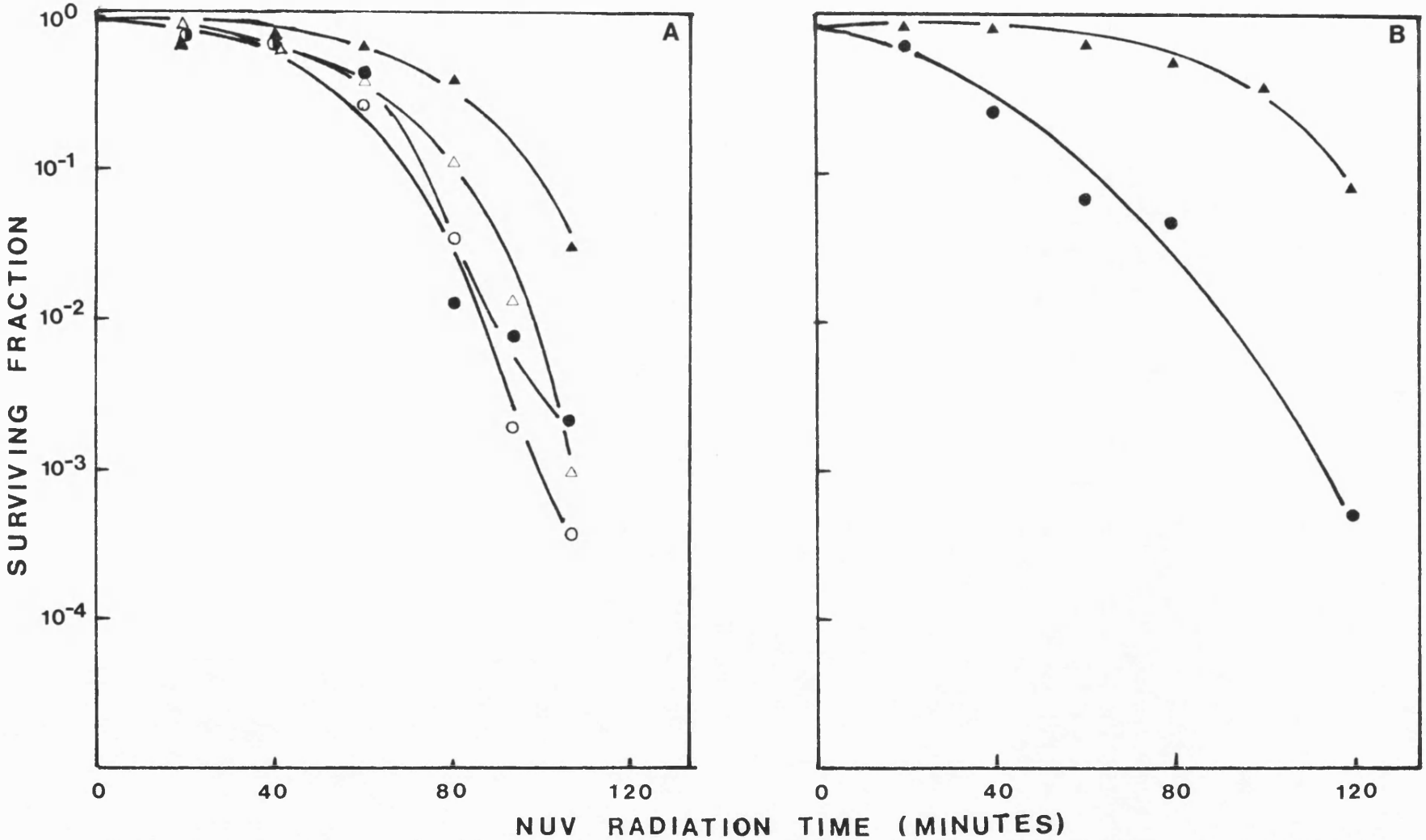


Figure 29: Survival curves for (A) *E. coli* UMI and (B) *E. coli* CSH7 after near-UV irradiation with pretreatment (○, △, A only) or without pretreatment (●, ▲) with catalase in the plating media (△, ▲) and without catalase (○, ●).

uvrA mutation does not influence the sensitivity of cells to broad-band near-UV radiation, whilst the *nur* mutation increases sensitivity to near-UV radiation (Tuveson, 1980). Also, Sammartano and Tuveson (1984) demonstrated that added catalase protects cells from near-UV radiation even if the cells have initially very different near-UV radiation sensitivities. They showed that added catalase does not protect against inactivation by far-UV radiation, confirming that near-UV radiation inactivates by a different mechanism than far-UV radiation.

Assays for Hydrogen Peroxide Scavenging Ability

The results in figs. 26-29 do not, however, explain the high sensitivity of exponential phase B/r to near-UV radiation. The observation that the sensitivity of B/r to near-UV radiation is decreased by either 30 μM H_2O_2 pretreatment or the addition of exogenous catalase suggests that exponential phase B/r may be deficient in constitutive enzymes whose function is replaced by induced enzymes or exogenous catalase. The absence of a similar extreme sensitivity of exponential phase B/r to 5 mM H_2O_2 would indicate that factors other than H_2O_2 are also involved in sensitivity to near-UV radiation. However, to ensure that the sensitivity of B/r to near-UV radiation was not due to a lack or decreased level of enzymes that degrade H_2O_2, cells were assayed for H_2O_2 scavenging ability.

The assay used to assess H_2O_2 scavenging ability utilizes the formation of a yellow coloured complex between the reagent titanium oxysulphate ($TiOSO_4$) and available H_2O_2. The assay measures the loss of H_2O_2 and thereby, indirectly, the H_2O_2 scavenging ability of the cells. Reference to the assay as a catalase assay in particular is avoided as the assay shows loss of H_2O_2 and is not specific for catalase activity. The results are presented as the mean of three optical density readings for

each point. The variation between OD_{405} readings for unpretreated, exponential phase B/r is shown in fig. 2, Appendix A.

The results for assays of H_2O_2 scavenging ability obtained with *E. coli* K12 AB1157, *E. coli* B/r and *E. coli* UMI are shown in fig. 30. The catalase mutant UMI exhibited no measurable ability to remove H_2O_2 either with or without pretreatment. Both K12 AB1157 and B/r showed very similar activities without pretreatment and both showed similar increased activity after pretreatment with 30 μM H_2O_2. The H_2O_2 scavenging ability of UMI is also shown compared to that of its parent strain CSH7 (fig. 31). CSH7 also showed increased H_2O_2 scavenging ability after H_2O_2 pretreatment although it was less marked that seen with either K12 AB1157 or B/r.

A single experiment was carried out to see whether pretreating cells with near-UV radiation would induce increased H_2O_2 scavenging ability. *Escherichia coli* K12 AB1157 cells were grown in 100 ml defined medium for 75 min. The growing culture was then divided into four 25 ml aliquots, centrifuged (MSE Chilspin centrifuge, MSE, Crawley, Sussex; 3500 rpm, 15 min) and resuspended in 10 ml M9 salts solution. A 5 ml portion was irradiated for 150 sec in position 7 in the black-light blue lamp-box. The remaining 5 ml was used as an unirradiated control. After irradiation, the 5 ml samples were added to two 50 ml volumes of prewarmed defined medium and grown for a further 60 min. Cells were then harvested and assayed for H_2O_2 scavenging ability as before.

The results of this experiment are shown in fig. 32. Exponential phase *E. coli* K12 AB1157 showed no measurable H_2O_2 scavenging ability without pretreatment in this experiment. Cells pretreated with 150 sec near-UV radiation during growth did however show increased H_2O_2 scavenging ability. This preliminary result indicates that protection against

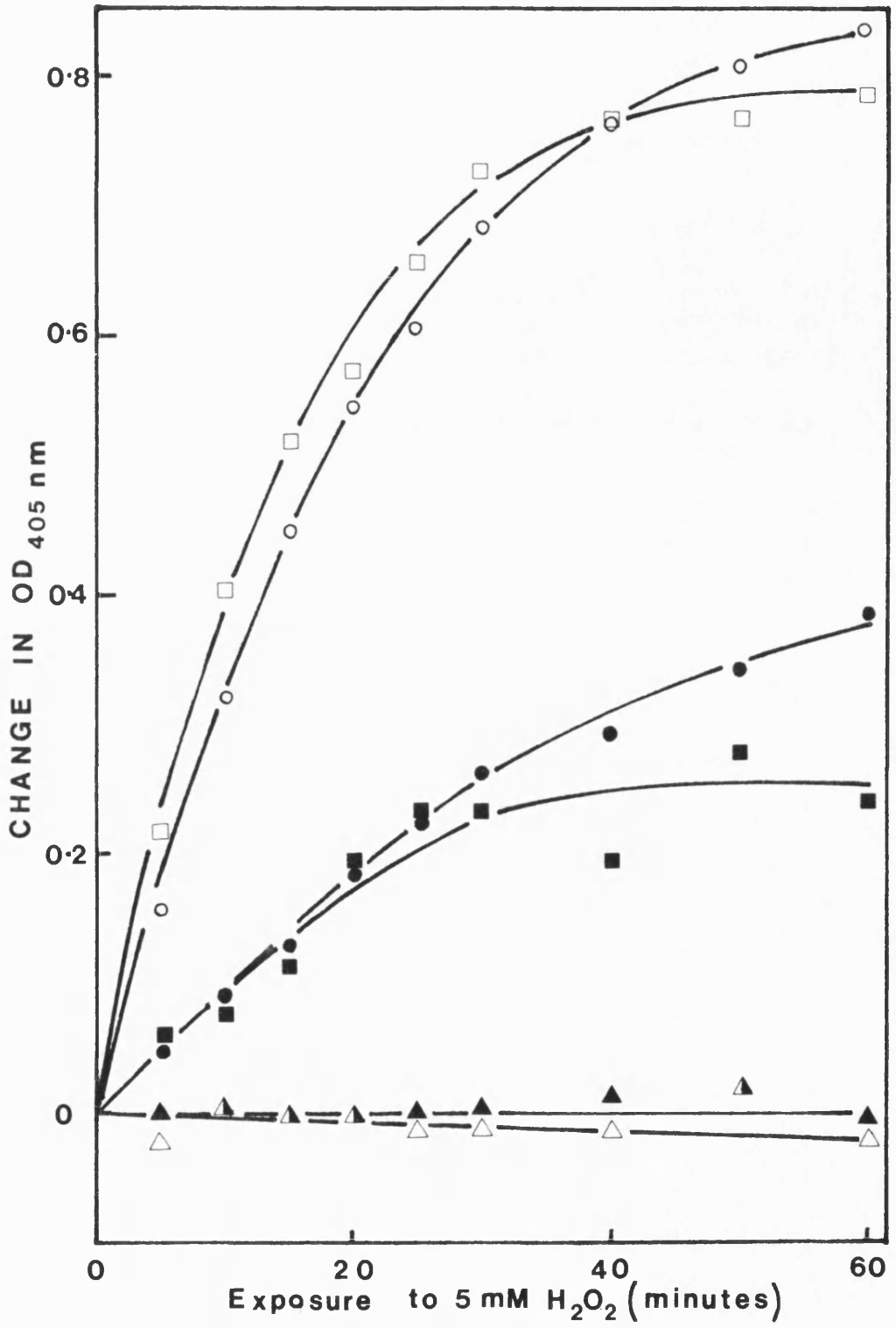


Figure 30: The H_2O_2 scavenging ability of *E. coli* K12 AB1157 (□,■), *E. coli* B/r (○,●) and *E. coli* UMI (△,▲) challenged with 5 mM H_2O_2, with pretreatment (□,○,△) and without pretreatment (■,●,▲).

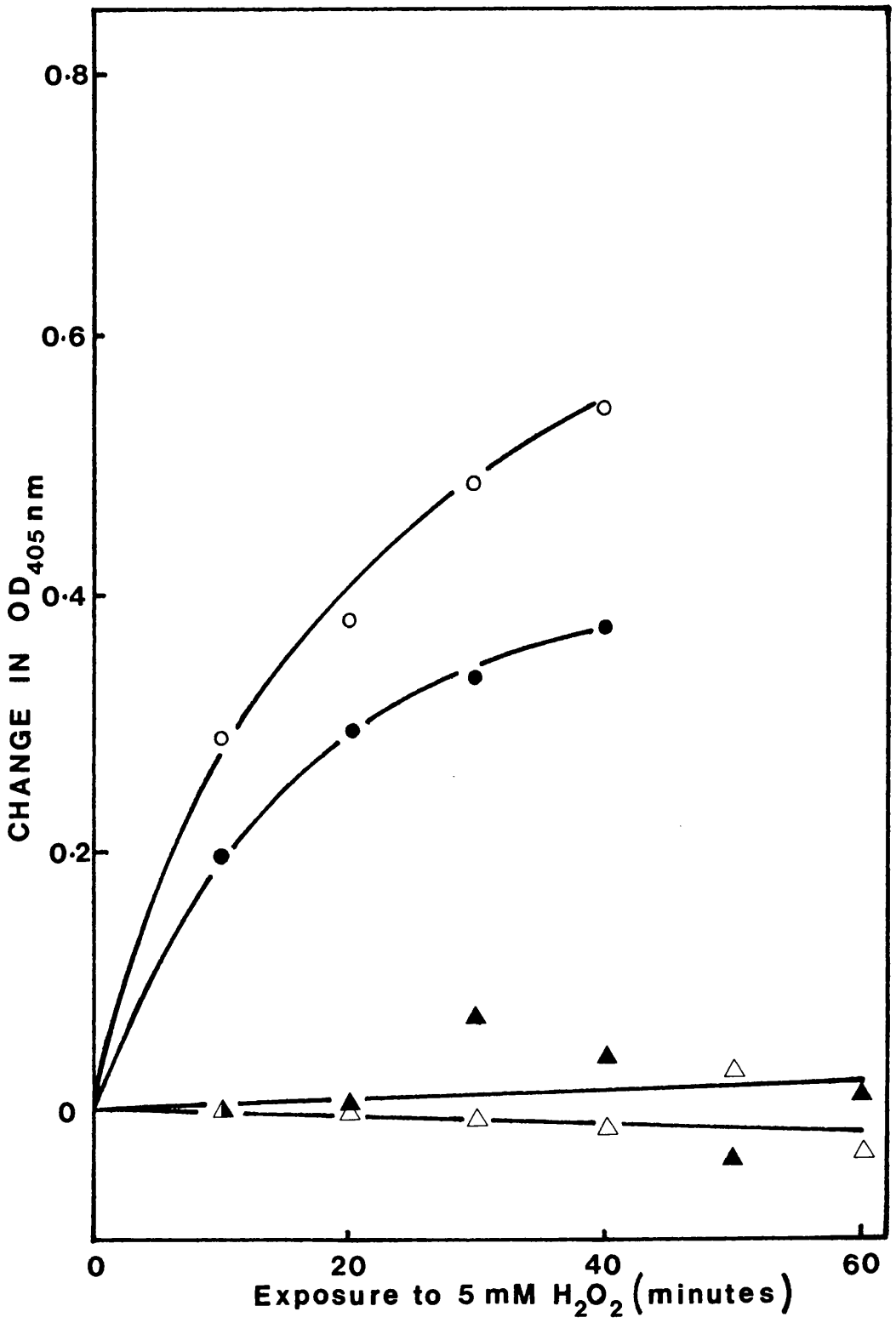


Figure 31: The H_2O_2 scavenging ability of *E. coli* UMI (△,▲) and *E. coli* CSH7 (○,●) challenged with 5 mM H_2O_2, with pretreatment (△,○) and without pretreatment (▲,●).

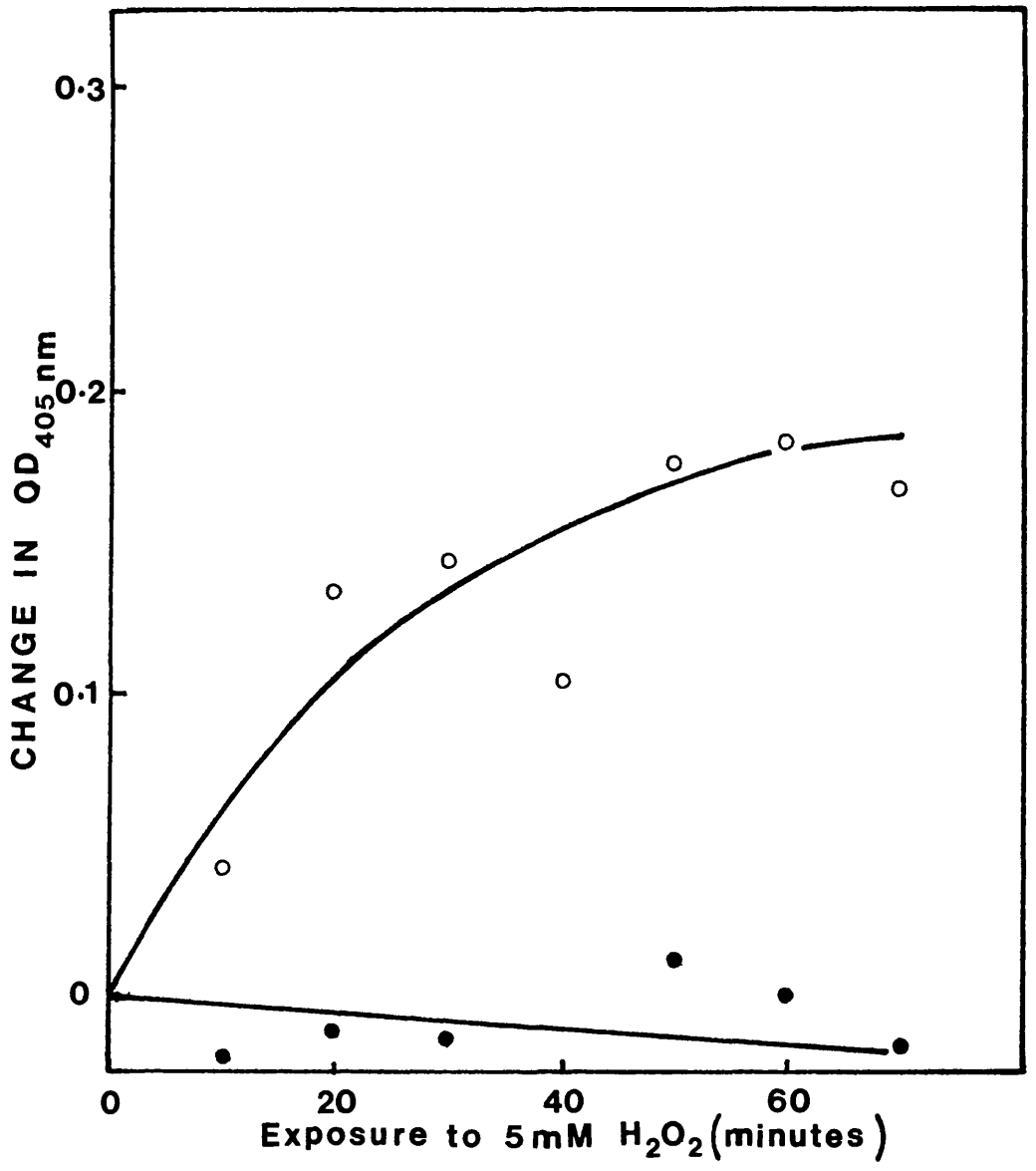


Figure 32: The H_2O_2 scavenging ability of *E. coli* K12 AB1157 challenged with 5 mM H_2O_2 with 150 sec. near-UV pretreatment (O) and without pretreatment (●).

oxidative damage is induced by pretreatment with near-UV radiation. This further confirms the findings of Tyrrell (1985) that suggest a common protective mechanism against H_2O_2 and near-UV radiation damage. It is not clear why the unpretreated cells show no measurable H_2O_2 scavenging ability in this experiment, whilst in the 30 μM H_2O_2 pretreatment experiments (fig. 30) activity was seen with the unpretreated cells. It may be that the method used to obtain cells for pretreatment suppresses endogenous catalase so that no activity is seen. However, conclusions cannot be drawn from this single, preliminary result.

The Effect of Growth Medium on the Inducible Response

All the data present so far in Section I of results were obtained with cells grown in defined media. It has been reported that the sensitivity of *Escherichia coli* cells to H_2O_2 is affected by factors that modify the amount of catalase sythesized by the cells; one such factor is the composition of the culture medium in which cells are grown (Yoshphe-Purer and Henis, 1976). In order to examine whether or not the inducible response for both H_2O_2 and near-UV radiation were affected by the growth medium, the experiments represented in figs. 20-25 were repeated using *E. coli* K12 AB1157 grown in nutrient broth (Oxoid) instead of defined media. Cells were pretreated with 30 μM H_2O_2 and harvested as previously described before challenging with either 5 mM H_2O_2 or near-UV radiation. The survival curves from these two experiments are shown in fig. 33A and B.

The survival curves obtained after pretreatment of exponential phase K12 AB1157 and challenge with 5 mM H_2O_2 (fig. 33A) show an induced response. The response induced by pretreatment with 30 μM H_2O_2 and that of unpretreated cells was the same as that seen when K12 AB1157 was grown and pretreated in defined media (fig. 20). However, when cells growing in

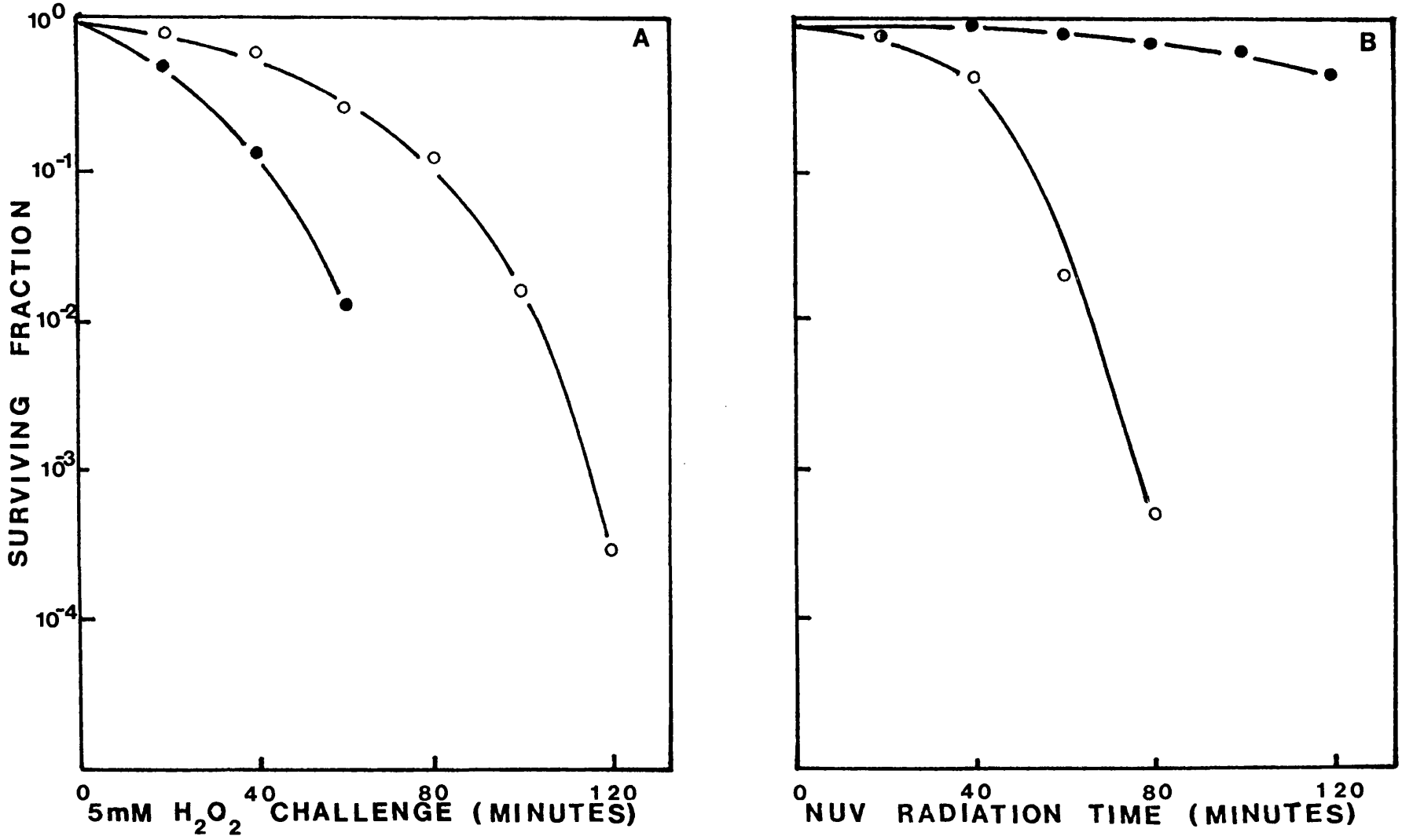


Figure 33: Survival curves for *E. coli* K12 AB1157 after (A) 5 mM H_2O_2 challenge and (B) near-UV irradiation, with 30 µM H_2O_2 pretreatment (○) and without pretreatment (●). Cells were grown in Oxoid nutrient broth.

nutrient broth were pretreated and challenged with near-UV radiation (fig. 33B) the result was the opposite of that seen previously (fig. 23A). Unpretreated cells were resistant to near-UV radiation showing approximately 0.5 log kill after 120 min irradiation, but cells that had received pretreatment showed increased sensitivity to near-UV radiation with greater than 3 log kill after 80 min irradiation.

Generally, cells grown in rich media have higher catalase levels than cells grown in defined media (Yoshpe-Purer and Henis, 1976). If this is so, then it is not apparent from the response of nutrient broth grown cells to 5 mM H_2O_2 challenge (fig. 33A). The response of unpretreated cells is the same as unpretreated cells grown in defined medium (fig. 20). The response of unpretreated cells to near-UV radiation (fig. 33B) shows that nutrient broth grown cells are considerably more resistant to near-UV radiation than defined medium grown cells. However, with nutrient broth grown cells, pretreatment with 30 µM H_2O_2 sensitizes the cells to near-UV radiation rather than increasing resistance. From the data presented here, it is unclear as to why this should occur. It could be that mechanisms other than those related to H_2O_2 induced damage are important under these experimental conditions.

Effect of Growth Phase

Catalase levels are also known to be affected by the phase of growth of cells (Yoshpe-Purer and Henis, 1976; Finn and Condon, 1975). Therefore, additional experiments were carried out to examine the effect of catalase added to the plating medium on the survival of stationary phase cells after near-UV irradiation. Stationary phase cells were obtained by inoculating 100 ml prewarmed defined media with 1 ml of a cell culture that had been grown overnight. This culture was then grown for 24 h before harvesting.

The results obtained for K12 AB1157 and B/r are shown in fig. 34A and B. A slight increase in survival was seen with both strains when catalase was added to the plating medium. A similar near-UV radiation sensitivity was shown by K12 AB1157 in both exponential and stationary phases of growth (figs. 23A and 34A) whilst B/r was much more resistant to near-UV radiation when in stationary phase as compared to exponential phase (figs. 23B and 34B).

The survival curves for SR385 show that added catalase protects stationary phase cells from near-UV inactivation (fig. 35A). However, stationary phase cells appeared to be more sensitive to near-UV radiation than exponential phase cells (figs. 24 and 35A). With UMI (fig. 35B) stationary phase cells were more resistant to that seen with exponential phase cells (fig. 25A) particularly at higher fluences. Again added catalase was seen to give additional protection.

It has been reported that the phase of growth of cells affects their near-UV radiation sensitivity and it is generally accepted that cells in exponential phase are more sensitive than stationary phase cells (Peak, 1970; and see discussion in the general introduction). However, differences have been reported. Kelland *et al.* (1984b) have reported that whilst B/r is more sensitive in exponential phase, the opposite is true for SR385 and to a lesser extent K12 AB1157. The results presented here (figs. 34 and 35) confirm this report.

Discussion

The data presented in this section of results show that firstly, pretreatment with low non-lethal levels of H_2O_2 increases the resistance of cells to subsequent H_2O_2 challenge. Secondly, they show that this pretreatment also confers protection against subsequent near-UV radiation

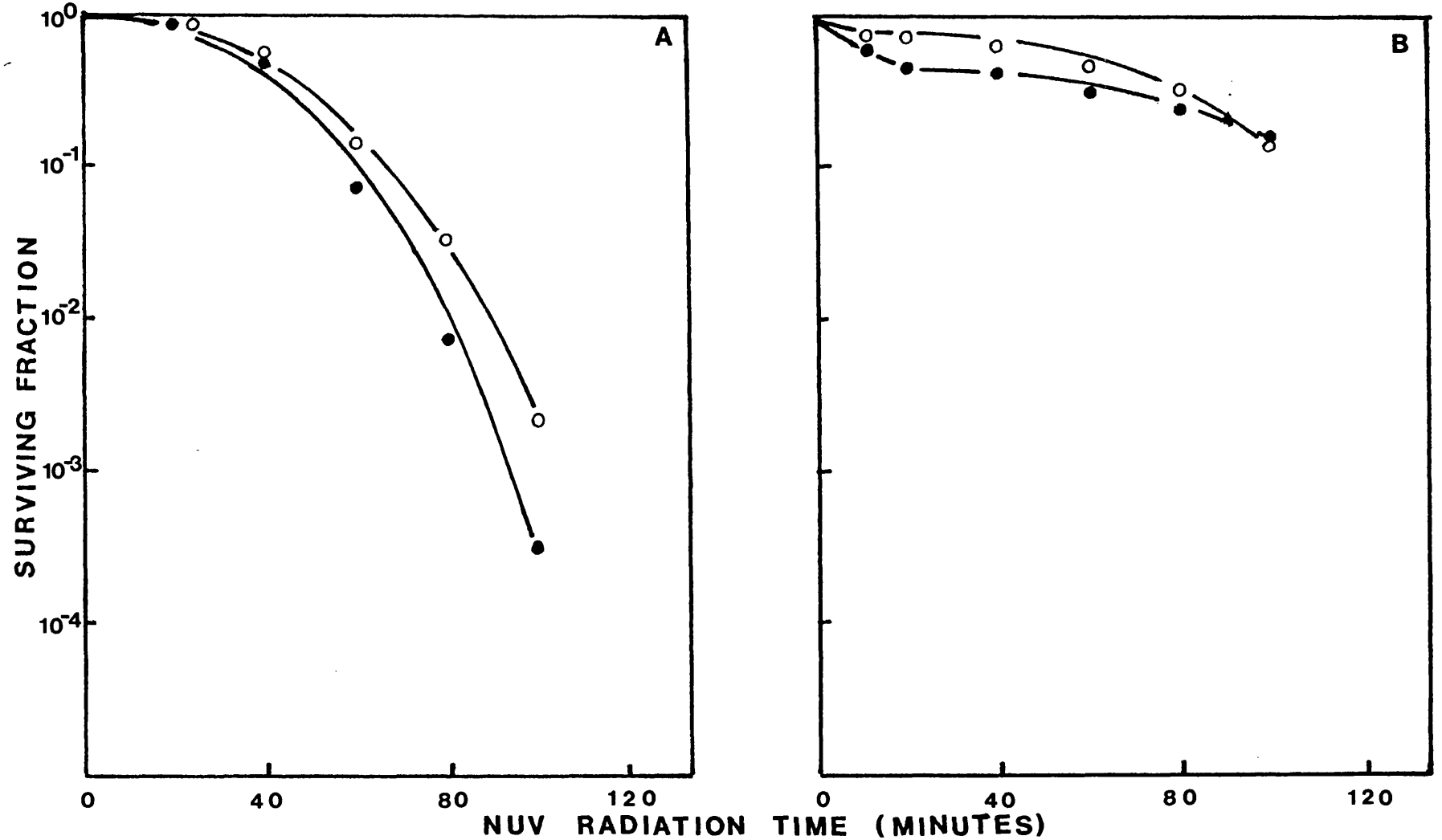


Figure 34: Survival curves for stationary phase (A) *E. coli* K12 AB1157 and (B) *E. coli* B/r after near-UV irradiation with catalase in the plating medium (o) and without catalase (●).

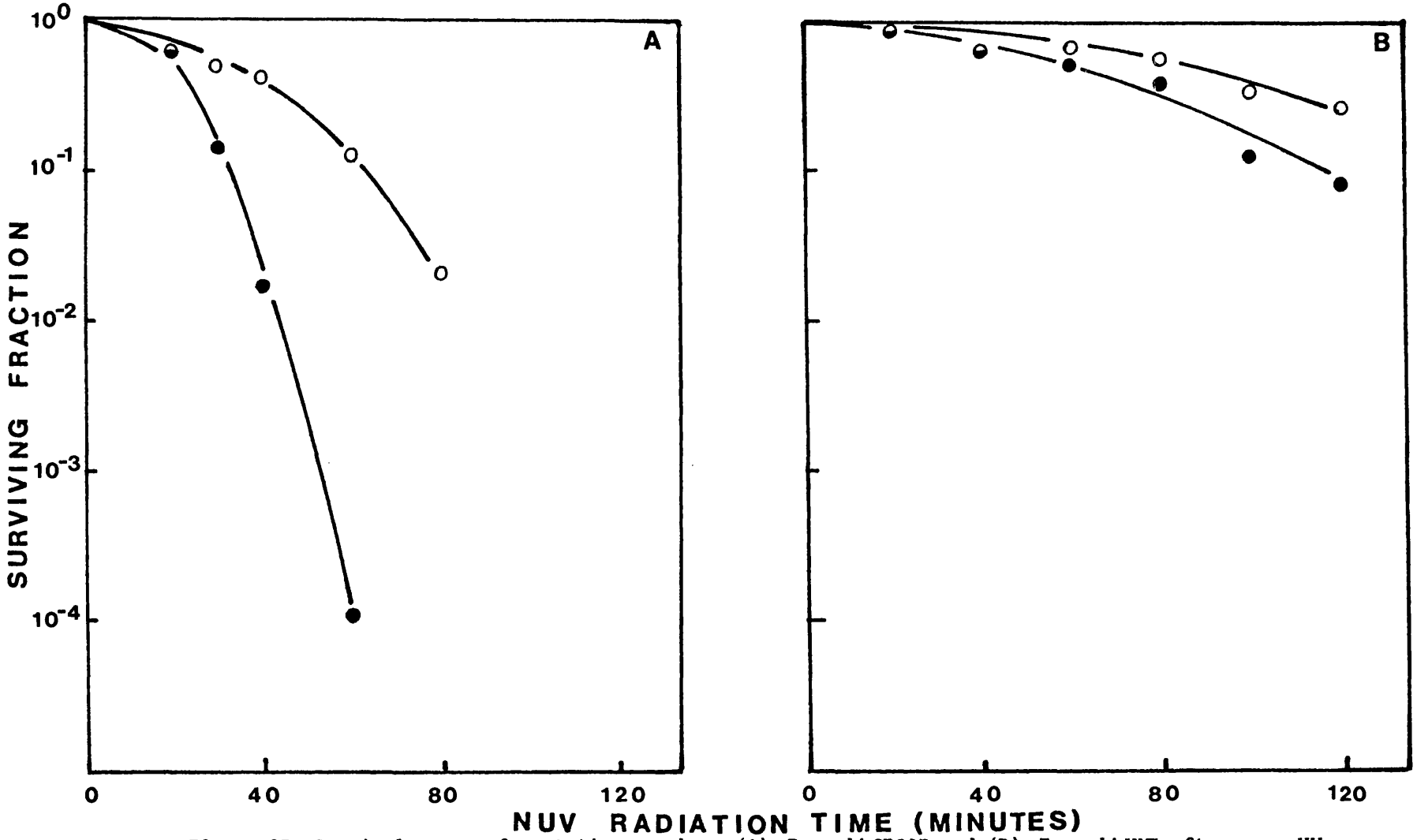


Figure 35: Survival curves for stationary phase (A) *E. coli* SR385 and (B) *E. coli* UMI after near-UV irradiation, with catalase in the plating medium (O) and without catalase (●).

challenge and thirdly, that the addition of catalase to the post-irradiation plating medium protects cells from near-UV radiation inactivation.

The results obtained from pretreating cells with 30 μM H_2O_2 and challenging with 5 mM H_2O_2 (figs. 20-22) support the observations of Demple and Halbrook (1983) and Tyrrell (1985) that resistance to H_2O_2 toxicity can be induced in *E. coli*. This response was seen with K12 AB1157 (fig. 20), B/r (fig. 21A), SR385 (fig. 21B) and CSH7 (fig. 22B). The effect was most marked with K12 AB1157 and B/r and less marked with SR385, especially at longer H_2O_2 exposures. The response with CSH7 was variable, although induced protection was always seen; in some experiments (e.g. fig. 22B) the induced response was more apparent after large H_2O_2 exposures; with other experiments (see Table 3, Appendix B), a greater induced protection was seen at the onset of challenge. This variability may be due in part to CSH7 being particularly sensitive to small variations in experimental procedure. The catalase deficient mutant, UMI, showed no inducible response as predicted (fig. 22A), although the degree of sensitivity to H_2O_2 in the unpretreated cells was less than might be expected. With this strain, pre-exposure to 30 μM H_2O_2 did not enhance the resistance of cells to 5 mM H_2O_2.

The results obtained when pretreated and unpretreated cells were exposed to near-UV radiation (figs. 23-25) were again in agreement with published data (Sammartano and Tuveson, 1985: Tyrrell, 1985). However, these results were not entirely consistent with theoretical considerations. Firstly, the increased resistance seen with K12 AB1157 (fig. 23A) was very slight and, unlike the response seen with H_2O_2 challenge, a rapid increase in sensitivity was seen at higher fluences. These results do, however, confirm findings of Tyrrell (1985). Using the same experimental conditions

and *E. coli* K12 AB1157, Tyrrell found that H_2O_2 pretreatment induced only a small degree of protection against monochromatic (334 and 365 nm) radiation. Secondly, although induced protection was seen with K12 AB1157 (fig. 23A), B/r (fig. 23B), SR385 (fig. 24) and CSH7 (fig. 25B), unpretreated exponential phase B/r showed greatly increased sensitivity to near-UV radiation (fig. 23B), contrasting to the similar responses shown by these four strains after challenge with 5 mM H_2O_2. Finally, the apparent resistance of UMI to near-UV radiation was also unpredicted, although, as was seen with H_2O_2, additional protection could not be induced by pretreatment with 30 μM H_2O_2.

There are several possibilities that may help to explain these inconsistancies. Induced resistance has been reported in *E. coli* (Demple and Halbrook, 1983: Tyrrell, 1985: Sammartano and Tuveson, 1984) and in *S. typhimurium* (Christman *et al.*, 1985). Adaptation to H_2O_2 involves the induction of 30 proteins some of which have been identified as catalase, manganese-containing superoxide dismutase, glutathione reductase and a novel alkyl hydroperoxide reductase (Christman *et al.*, 1985). It has also been shown that H_2O_2 inducible proteins overlap with proteins that are induced by other stresses, such as heat shock (Morgan *et al.*, 1986; Van Bogelen *et al.*, 1987). Therefore the enzymes induced by pretreatment with H_2O_2 may give maximal protection against lethal challenge with H_2O_2 but less effective protection against near-UV irradiation. However, the induced responses do overlap as shown by the results in figs. 20-25 and fig. 32 and those reported by Tyrrell (1985).

Further differences may arise from different modes of production of H_2O_2. When cells are challenged with 5 mM H_2O_2, they are immediately exposed to a high concentration of H_2O_2. When cells are irradiated, however, H_2O_2 generated during irradiation is likely to be present at low

concentrations, with cells gradually accumulating damage until a toxic level is reached. It has been shown by Imlay and Linn (1986) that H_2O_2 killing has two different modes of action at different H_2O_2 concentrations; mode 1 is maximal at 1-2 mM H_2O_2 and mode 2 occurs at higher concentrations. These different modes of action may be reflected in the responses of cells to H_2O_2 (5 mM) and near-UV radiation (gradually accumulating, low level H_2O_2).

The different modes of H_2O_2 action may also partly help to explain the apparent resistance of UMI to near-UV radiation. Although UMI, *katE, katG*, is catalase deficient, the damage caused by near-UV mediated H_2O_2 may be repaired by repair enzymes and is thus prevented from accumulating to a toxic level. Both *xthA* (Demple and Halbrook, 1983) and *recA* (Carlsson and Carpenter, 1980) have been shown to be important against H_2O_2 damage and evidence has been presented to show that catalase has only a minor role in protection against near-UV radiation damage (Eisenstark and Perrot, 1987). Furthermore, Kramer and Ames (1987) have suggested that H_2O_2 is not important in the effects of near-UV radiation. However, this conclusion was based on data from experimental procedures designed to approximate environmental conditions (exposure to a broad-band near-UV radiation source with low fluence rate, approximately 35 Wm^{-2}, over several hours). Under these conditions H_2O_2 may not accumulate sufficiently to produce lethal effects.

The results presented in figs. 26, 27 and 29 confirm the findings of Sammartano and Tuveson (1984) that the addition of catalase to the post-irradiation plating medium protects cells against near-UV radiation inactivation. The effect was seen with all five strains tested, regardless of the near-UV radiation sensitivity of unpretreated cells. Sammartano and Tuveson (1984) suggest that H_2O_2 generated during near-UV radiation

exposure gradually decays into reactive species. One such likely species would be superoxide radicals. However, the addition of superoxide dismutase to the plating medium (10-100 µg/ml) had very little effect on the survival of K12 AB1157 (fig. 28) indicating that this particular species may not be involved.

It is possible that cells undergoing near-UV irradiation are able to repair the DNA damage that occurs at low fluences, but at higher fluences, cells have accumulated damage and are susceptible to additional attack by near-UV radiation mediated H_2O_2. Catalase in the plating medium could, then, act by removing any free H_2O_2 before it "fixes" and enhances sublethal damage present in the cells. The susceptibility of cells to post-irradiation stress has been previously show in *E. coli*. Kelland *et al.* (1983 a and b) have shown that broad-band near-UV irradiated *E. coli* cells are sensitive to high salt concentrations (220 mM) in the plating medium and that cells will recover from this sensitivity if held in a complex post-irradiation recovery medium. This would indicate that cells are able to recover from near-UV radiation induced damage under favourable post-irradiation conditions. Exogenously added catalase may help in this respect. The addition of catalase to the post-irradiation plating medium also slightly enhanced recovery of stationary phase cells (figs. 34 and 35). Since this effect is seen in all strains tested and in both exponential and stationary phases of growth, it would suggest that exogenously added catalase has a non-specific role in protection and is not merely acting by replacing deficient enzyme levels within the cells.

The results shown in fig. 30 compare the H_2O_2 scavenging ability of K12 AB1157, B/r and UMI with and without 30 µM H_2O_2 pretreatment. The results presented are entirely consistent with the survival curves for H_2O_2 sensitivity shown in figs. 20-22. Both K12 AB1157 and B/r show similar

H_2O_2 scavenging ability in the unpretreated state just as their survivals after 5 mM H_2O_2 challenge were similar. Likewise they show a large inducible increase in H_2O_2 scavenging ability after H_2O_2 pretreatment. This also reflects the increased survival of cells after pretreatment (figs. 20 and 21A). UMI showed no measurable H_2O_2 scavenging ability either with or without pretreatment, again reflecting the survival response to H_2O_2. CSH7 (fig. 31) showed a response similar to K12 AB1157 and B/r with H_2O_2 scavenging ability present before pretreatment and increased ability after pretreatment. The induced response was not so great as that seen with K12 AB1157 and B/r, which may reflect the variable inducible response to 5 mM H_2O_2 seen in CSH7.

When the H_2O_2 scavenging abilities of K12 AB1157, B/r and UMI (fig. 30) are compared to their near-UV radiation sensitivities (figs. 23A, 23B, 25A) there is less correlation. Although the response of pretreated cells to near-UV radiation is reflected by their H_2O_2 scavenging ability, the response of unpretreated cells is not. B/r, which shows greatest sensitivity to near-UV radiation, does not have the lowest H_2O_2 scavenging ability whilst UMI, which shows similar near-UV radiation sensitivity to K12 AB1157, does not share a similar H_2O_2 scavenging ability.

Since the high sensitivity of B/r to near-UV radiation can be reduced by either pretreating cells with 30 μM H_2O_2 (fig. 23B) or by the addition of exogenous catalase (fig. 26B), this would suggest that B/r is relatively deficient in constitutive enzyme(s) whose functions may be replaced by induced enzymes or exogenous catalase. However, the absence of extreme sensitivity of exponential phase B/r to H_2O_2 (fig. 21A) is not consistent with this explanation. It is unlikely that exponential phase B/r is lacking catalase, as the catalase deficient mutant UMI is more resistant to near-UV radiation under the same conditions (fig. 25A). Likewise the

sensitivity of B/r cannot be related non-specifically to peroxide scavenging ability as the assay using complexation with $TiOSO_4$ shows a considerable increase over the very low level exhibited by UMI (fig. 30). Also, B/r shows the same response in the H_2O_2 scavenging assay as that shown by K12 AB1157 (fig. 30) despite being consistently more near-UV radiation sensitive than K12 AB1157 in the unpretreated state (figs. 23A and B).

An alternative hypothesis arises from the work of Thiam and Favre (1984). They reported that the near-UV radiation induced growth delay in exponential phase *E. coli* B/r WP2 was approximately four times that of exponential phase *E. coli* K12 AB1157 and that correspondingly higher levels of ppGpp were found in B/r WP2. This induction of ppGpp synthesis results in inhibition of stable RNA synthesis and therefore inhibition of protein synthesis. If exponential phase B/r is sensitive to near-UV radiation induced stringent control, it is possible that the synthesis of H_2O_2 scavenging enzymes is inhibited by the early part of near-UV irradiation. This would render the cells particularly sensitive to H_2O_2 produced during irradiation or H_2O_2 in the plating medium. Furthermore, the induction of H_2O_2 scavenging enzymes or the addition of exogenous catalase would be expected to enhance survival as has indeed been shown experimentally. Section 2 of the results will examine this hypothesis, in the context of growth phase effects, by using growth delay mutants (*relA*$^+$ - stringent control and *relA*$^-$ - relaxed control) and examining their relative sensitivities to near-UV radiation.

RESULTS: SECTION 2

The Effect of Phase of Growth on Near-UV Radiation Induced Sensitivity in *Escherichia coli*

One of the major findings in Section 1 of the results was that although both *E. coli* B/r and *E. coli* K12 AB1157 show similar responses to H_2O_2 challenge and show increased resistance to both H_2O_2 and near-UV radiation after H_2O_2 pretreatment, exponential phase B/r is considerably more sensitive to near-UV irradiation than exponential phase AB1157. However, with stationary phase cells, the opposite was seen, with stationary phase B/r being more resistant to near-UV radiation lethality than stationary phase AB1157. Although it has been reported by some workers that cells in the exponential phase of growth are more sensitive to near-UV radiation than stationary phase cells (Peak, 1970), other workers have reported that growth phase effects are strain dependent (Kelland *et al.*, 1984b) in agreement with the findings of Section 1 of the results. A general discussion of the effect of growth phase on near-UV radiation sensitivity was included in the Introduction.

This section of results examines growth phase variations with respect to near-UV radiation sensitivity of *E. coli* B/r and comparisons are made with the growth phase responses of *E. coli* K12 AB1157. In addition, the possible involvement of the stringent response in growth phase effects is examined using two pairs of isogenic *relA*$^+$/*relA*$^-$ strains.

For the experiments described in this section, some additional methods were used that were not described in the general methodology.

Methods

Growth Phase Experiments

Growth phase experiments were carried out to determine the near-UV radiation sensitivity of cells at different stages of growth. Cells were grown at 37°C in defined media plus glucose and the appropriate growth

supplements as described in the general methodology. Growing cultures were obtained by inoculating prewarmed media with 1 ml of a 24 h stationary phase culture. This gave an initial cell density of approximately 10^7 CFU/ml. Cells were harvested, at the appropriate times after the culture had been established, by membrane filtration and resuspended in M9 salts solution to give 10^7 CFU/ml. Cells were then irradiated and survival assessed on nutrient agar plates. The growth of the cultures was followed by optical density measurements (OD470) or by viable count throughout the sampling period. Normally samples were taken over an 8 h period. For sampling over longer periods (upto 24 h), one culture, A, was inoculated, as described above, 12 h before the start of an experiment and a second culture, B, was inoculated at the start of the experiment. Culture B was sampled for 0-12 h growth and culture A was sampled simultaneously for 12-24 h growth.

Extending Exponential Phase

The duration of exponential phase growth was extended by inoculating cultures with a 'low' cell number inoculum, thus allowing a longer period of growth before the culture reached saturation. The low cell number inoculum was achieved by either inoculating the culture with a small volume of cells (0.1 ml of 24 h stationary phase cells instead of the usual 1 ml) to give an initial cell density of 10^6 CFU/ml, or by inoculation with 1 ml of a x1000 dilution of 24 h stationary phase cells to give an initial cell density of 10^4 CFU/ml. For low cell numbers, 5 ml samples for irradiation were taken direct from the culture and briefly chilled on ice to arrest growth before irradiation.

Eliminating Lag Phase

The lag phase of growth was eliminated by using exponential phase cells rather than stationary phase cells as the inoculum. An exponential phase culture was obtained by inoculating prewarmed media with 1 ml 24 h stationary phase cells. This culture was grown at 37°C for 2 h and then 1 ml used to inoculate 100 ml fresh prewarmed media, giving an inoculum size of 10^5 CFU/ml. Growth was followed by viable count and samples were taken and irradiated as described above.

Growth Delay

Near-UV induced growth delay was used to test the *relA*$^+$/*relA*$^-$ phenotype of cells. Cultures were grown to mid-exponential phase (4 h). Cells were harvested by membrane filtration and resuspended in 10 ml M9 salts solution to give approximately $5x10^8$ CFU/ml. A 5 ml aliquot of this suspension was irradiated for 15 min (giving a sub-lethal dose) and the remainder used as an unirradiated control. After irradiation, the samples were transferred to 100 ml prewarmed defined growth media and growth was followed by optical density measurements.

Results

The results presented in this section were all obtained with cells grown in defined media with the appropriate growth supplements added (as described in the general methodology) and plated, after irradiation, to nutrient agar (Oxoid) plates.

Growth Phase Effects in *E. coli* B/r and *E. coli* K12 AB1157

Figure 36 shows a direct comparison of the near-UV radiation sensitivities of stationary phase *E. coli* B/r and *E. coli* K12 AB1157. After 120 min near-UV irradiation, stationary phase B/r exhibited less than one log cycle inactivation. The survival curve for stationary phase AB1157 showed an initial shoulder region of approximately 40 min followed by a rapid increase in sensitivity giving greater than 3 log kill after 120 min irradiation.

These results are the opposite of those shown in figs. 23A and B in Section 1 of the results, which were obtained with cells in the early exponential phase of growth (2.25 h). Therefore there must be a point or region of transition where intermediate near-UV radiation sensitivities are seen. As a preliminary study of this, survival curves were obtained for cells in the early-exponential (2 h), mid-exponential (4 h) and stationary (24 h) phases of growth. The results obtained are shown in fig. 37A and B. With both B/r (A) and AB1157(B), mid-exponential phase cells gave an intermediate degree of near-UV radiation-induced sensitivity.

Near-UV radiation sensitivity was then followed as a function of growth over an 8 h period. Prewarmed growth media was inoculated with stationary phase cells. Growth of the cultures was followed by optical density measurements and samples were irradiated for 100 min. The results are shown in fig. 38A and B. Results are plotted as surviving fraction against the number of hours of growth in defined media before sampling. The growth curves obtained from optical density readings of the cultures are also plotted to give a visual indication of the phase of growth of the cells. Figure 38A shows the growth phase response of B/r. Initially cells are resistant to near-UV radiation. This is followed by a rapid increase in sensitivity. The cells show greatest sensitivity after approximately

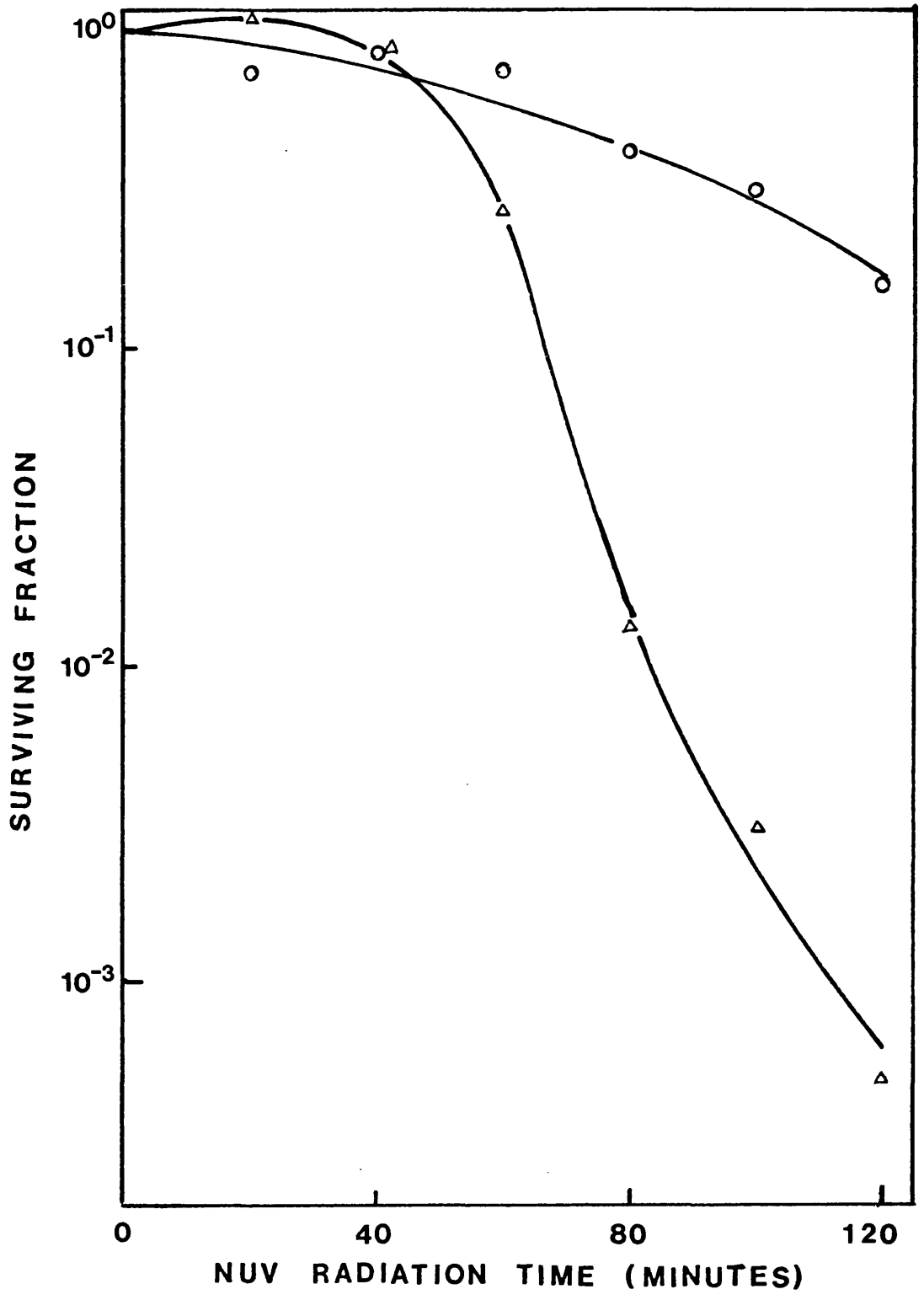


Figure 36: Survival curves for stationary phase (24 h) *E. coli* K12 AB1157 (△) and *E. coli* B/r (○).

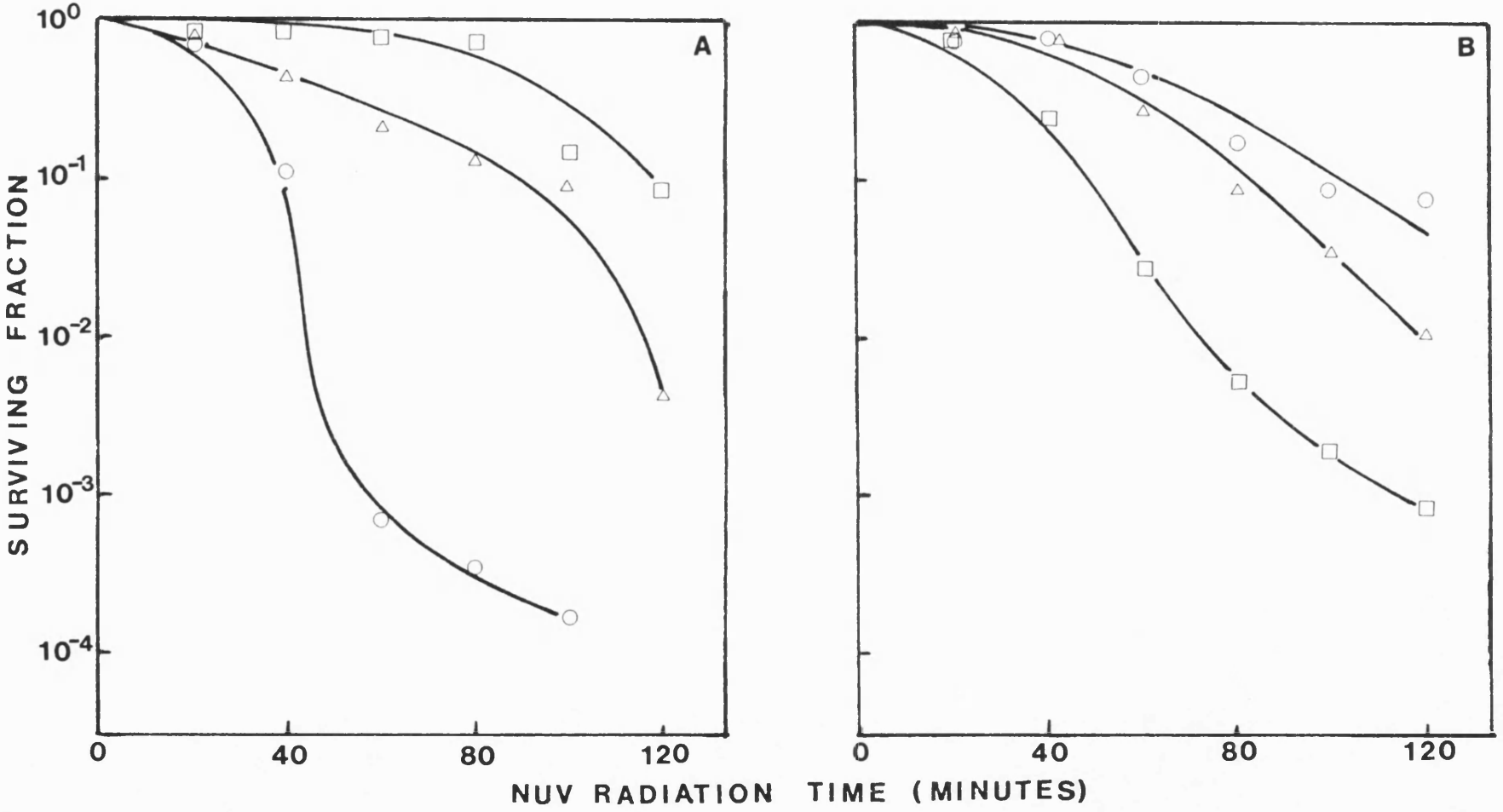


Figure 37: Survival curves for (A) *E. coli* B/r and (B) *E. coli* K12 AB1157 after 2 h (○), 4 h (△) and 24 h (□) growth in defined media.

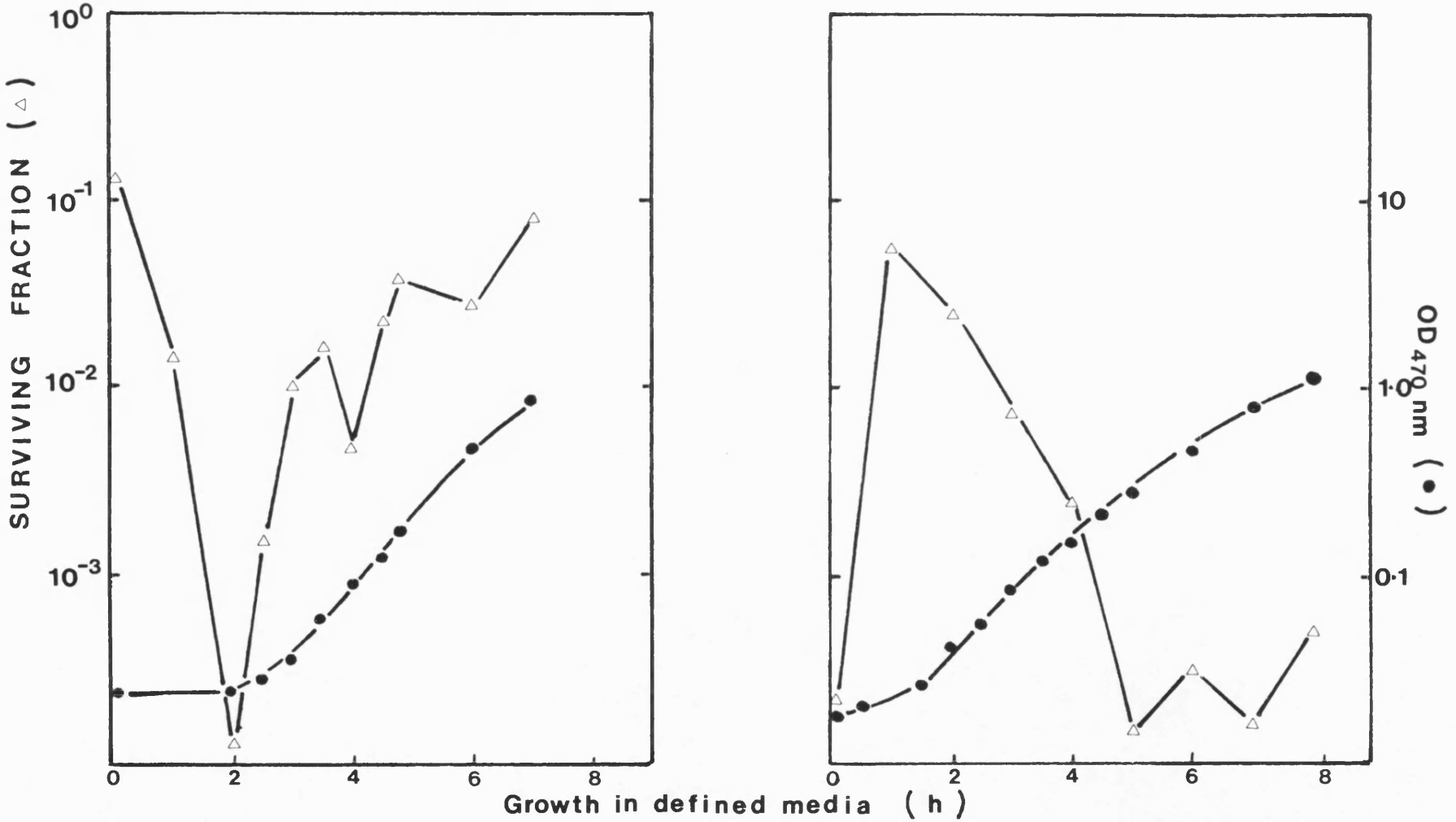


Figure 38: Surviving fractions (△) for (A) *E. coli* B/r and (B) *E. coli* K12 AB1157 after 100 min near-UV irradiation and optical densities (●) at 470 nm after growth in defined media for the number of hours shown on the abscissa.

2 h growth in defined media. Sensitivity to near-UV radiation decreased throughout exponential phase until a level of survival approaching that of the 0 h sample was reached after 7 h. With AB1157 (fig. 38B), almost the opposite was seen. Cells were initially sensitive but showed a rapid increase in resistance early in growth. This was followed by a steady decline in resistance during exponential phase.

Two interesting points arise from fig. 38. Firstly, the response of cells at the initiation of the new culture (0 h) is similar to that exhibited by 24 h cells (fig. 36). This is to be expected since cells taken from a 24 h culture were used as the inoculum. The cells sampled at 0 h would have had not time to adapt to their new environment before being harvested for irradiation, thus giving the same response as 24 h cells. Secondly, the most marked changes in near-UV radiation sensitivity occur during the lag and early exponential phases of growth with the response of cells returning to that of stationary phase cells towards the end of exponential phase.

In order to determine whether the response to near-UV radiation remained constant during stationary phase or whether similar fluctuations as seen during exponential phase were seen throughout the growth cycle, the experiment represented in fig. 38 was repeated but growth was followed for a 22 h period rather than an 8 h period. The results are shown in fig. 39 for B/r and fig. 40 for AB1157. For both B/r and AB1157, the results show that once stationary phase has been reached, there are no major fluctuations in near-UV radiation sensitivity.

Characterization of Growth Phase Effects in *E. coli* B/r

Since the most marked changes in response to near-UV radiation occur during exponential phase, a series of experiments was devised to

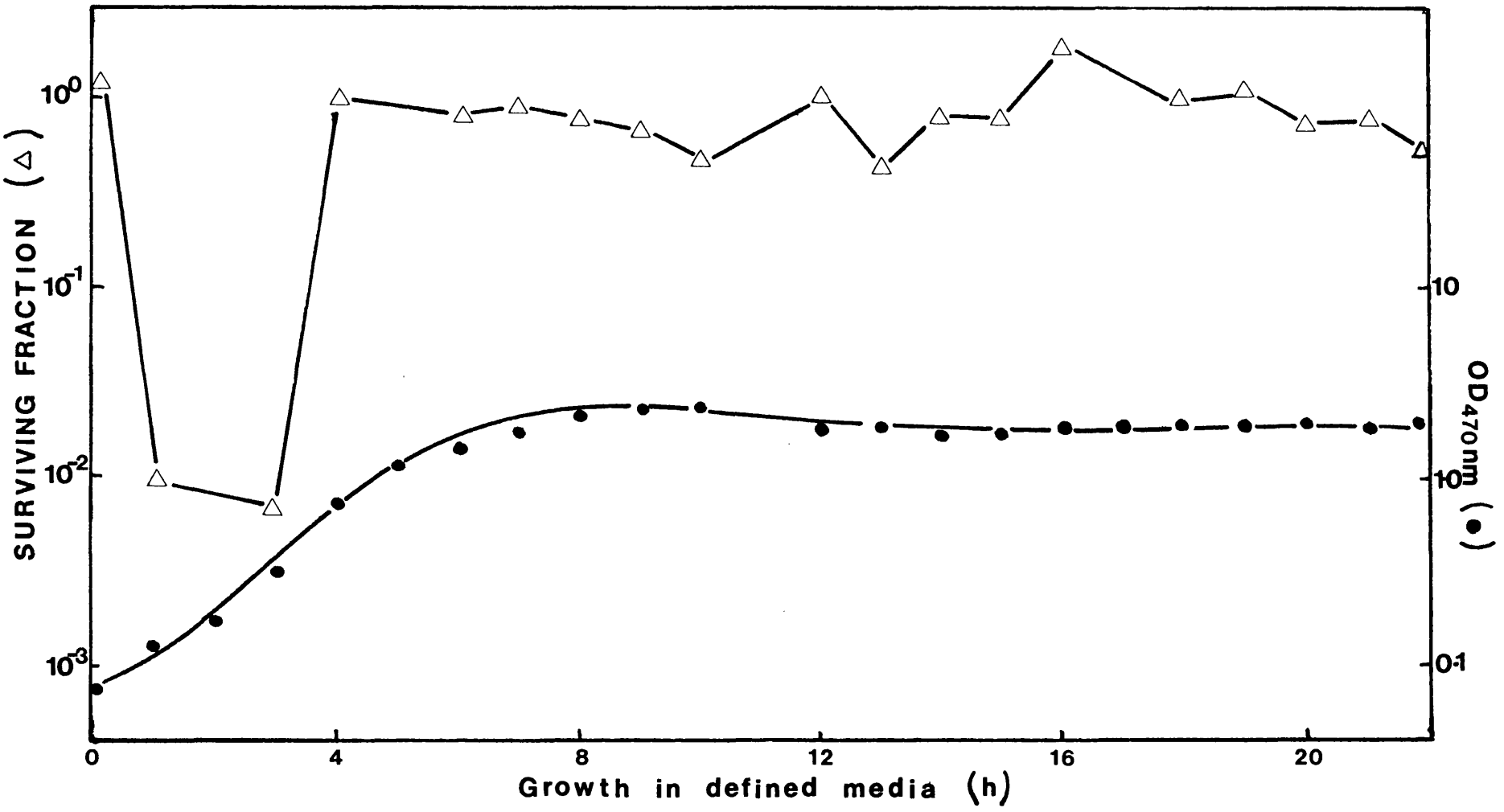


Figure 39: Surviving fractions (△) for *E. coli* B/r after 100 min near-UV irradiation and optical densitites (●) at 470 nm after growth in defined media for the number of hours shown on the abscissa.

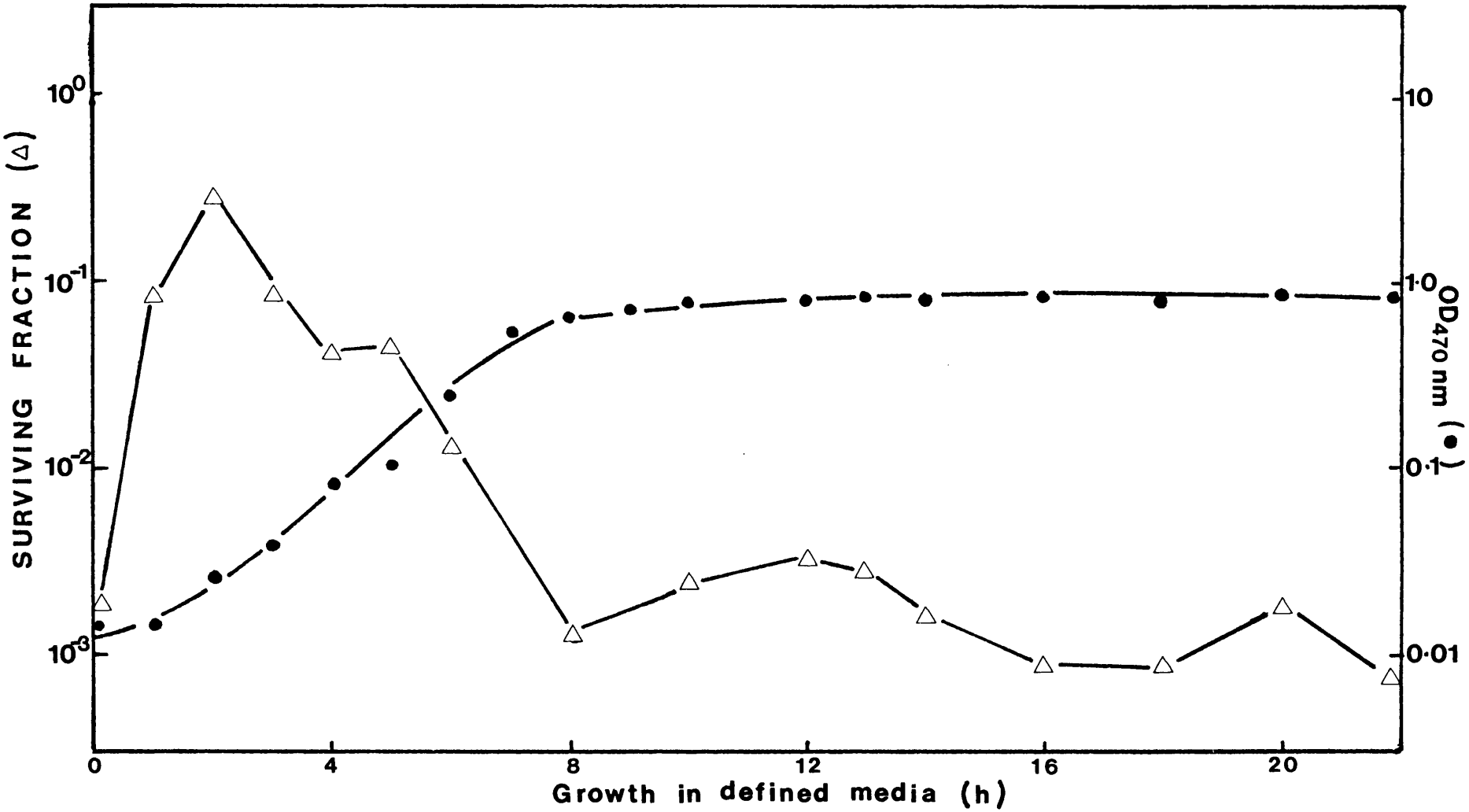


Figure 40: Surviving fractions (△) for *E. coli* K12 AB1157 after 100 min near-UV irradiation and optical densities (●) at 470 nm after growth in defined media for the number of hours shown on the abscissa.

investigate the nature of the response seen in exponential phase B/r. B/r was chosen since it is, firstly, phenotypically less complex than AB1157 and thus nearer a wild-type strain and, secondly, because the increase in sensitivity seen early in growth is large and well defined.

The period of greatest near-UV radiation sensitivity of B/r is seen after approximately 2 h growth (fig. 38A). This coincides with the beginning of exponential phase of growth, or alternatively, the end of lag phase. Since cells are both chemically and morphologically very different depending on their phase of growth, it firstly had to be determined whether the near-UV radiation sensitivity was associated with lag or exponential phase. The method employed to study this was based on extending the exponential phase of growth. If the near-UV radiation-induced sensitivity was associated with exponential phase, then by extending exponential phase the region of near-UV radiation sensitivity would also be extended. The method used to achieve this is shown in fig. 41. The 'normal' growth curve (closed symbols) was obtained from a growing culture produced by inoculating 100 ml prewarmed media with 1 ml stationary phase (24 h) cells. This gave an initial cell density of 10^7 CFU/ml. The 'extended' growth curve (open symbols) was obtained from a culture produced by inoculating 100 ml prewarmed media with 0.1 ml stationary phase cells giving an initial cell density of 10^6 CFU/ml. Figure 41 shows that after an initial lag period of approximately 1 h (a), there is a period of rapid exponential growth. For the normal growth curve this period (b), lasts for approximately 2.5 h. However, for the extended growth curve the exponential growth period (c) lasts approximately 4.5 h.

Thus, the time spent in exponential phase has been extended and it would be expected that any period of near-UV radiation sensitivity (or resistance) associated with early exponential phase would also be

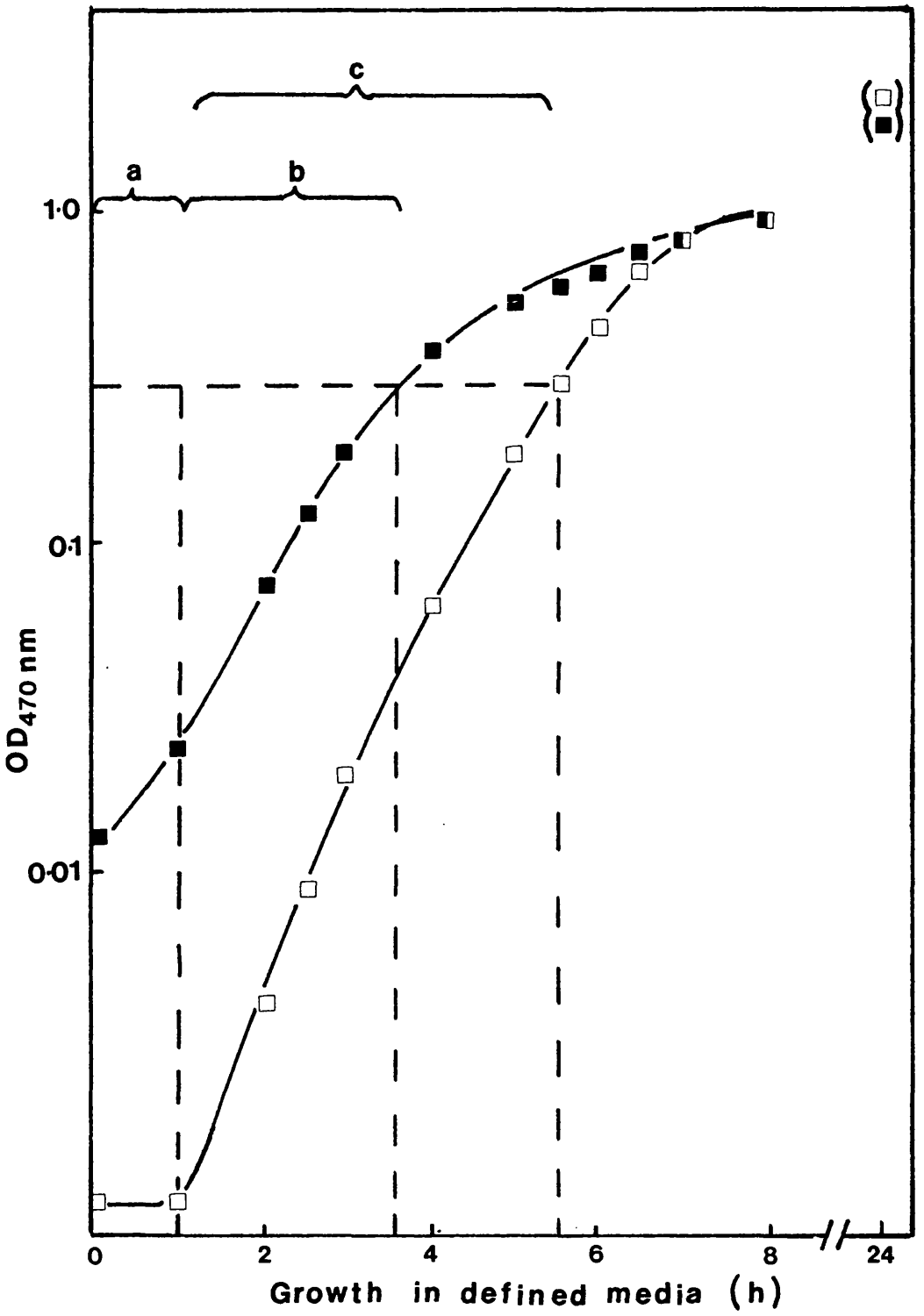


Figure 41: Growth curves obtained for *E. coli* B/r using 1 ml (■) or 0.1 ml (□) aliquots of 24 h stationary phase cells to incoluate 100 ml prewarmed growth media; a = lag phase; b = exponential phase for 1 ml inoculum; c = equivalent exponential phase period for 0.1 ml inoculum.

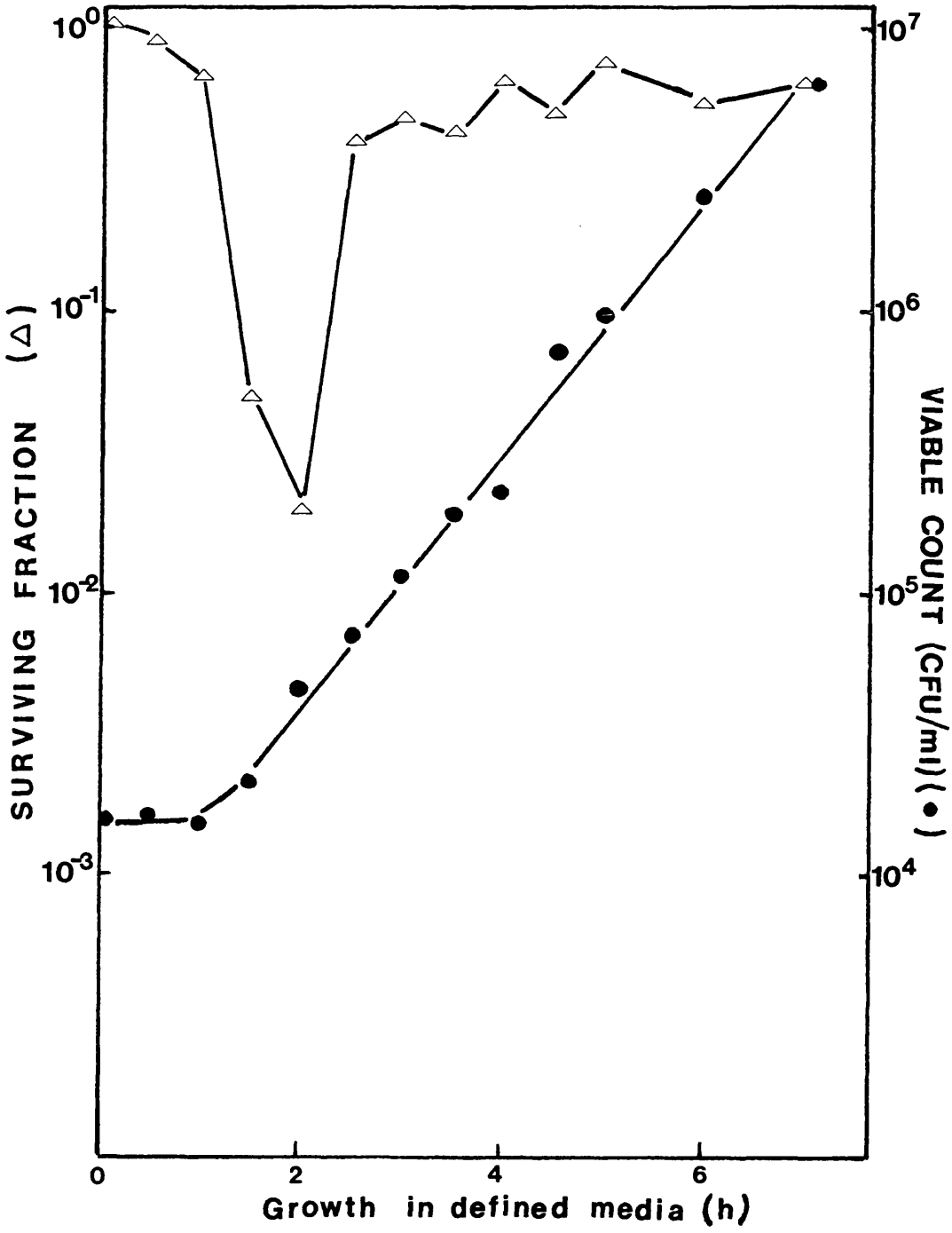


Figure 42: Surviving fractions (△) for *E. coli* B/r after near-UV irradiation for 60 min and viable counts (●) (CFU/ml) after growth in defined media for the number of hours shown on the abscissa: low cell inoculum.

increased. To examine whether or not this was in fact true, the experiment represented in fig. 38A was repeated but using a low cell inoculum, giving an initial cell density of 10^4 CFU/ml. The result is shown in fig. 42. The growth of the culture was followed by viable count since initially the optical density of the culture was too low to be read on the SP600 spectrophotometer. A comparison of growth curves obtained both by optical density readings and by viable count is included in Appendix A, fig. 3. In fig. 42 cells were irradiated for 60 min rather than 100 min, as shown in fig. 38A, resulting in a smaller dip in near-UV radiation sensitivity at 2 h. Nonetheless, the same overall profile is seen with a rapid increase in sensitivity at 2 h. However, although the length of exponential phase growth has been increased, the period of near-UV radiation sensitivity has not been extended. This suggests that the sensitivity is associated with lag phase rather than exponential phase growth.

This sensitivity may be due to the physiological state of lag phase cells. However, it is also possible that the dip in sensitivity at 2 h is an artifact of subculturing the cells. If the increased sensitivity was due to the state of cells in lag phase, then removing the lag phase should remove the increased sensitivity. If, on the other hand, the dip occurs in response to subculturing, then any subculturing process will produce the same effect at the same time after subculturing, *i.e.* 2 h. Therefore an experiment was performed in which the lag phase was eliminated. This was achieved by using growing cells as the inoculum. Cells were grown for 2 h in defined medium before being used to inoculate prewarmed media. Samples were taken for irradiation as before and growth was followed by viable count. The result is shown in fig. 43.

The growth curve (viable counts) (fig. 43) shows that the lag phase has been eliminated and that exponential growth was seen throughout the 4 h

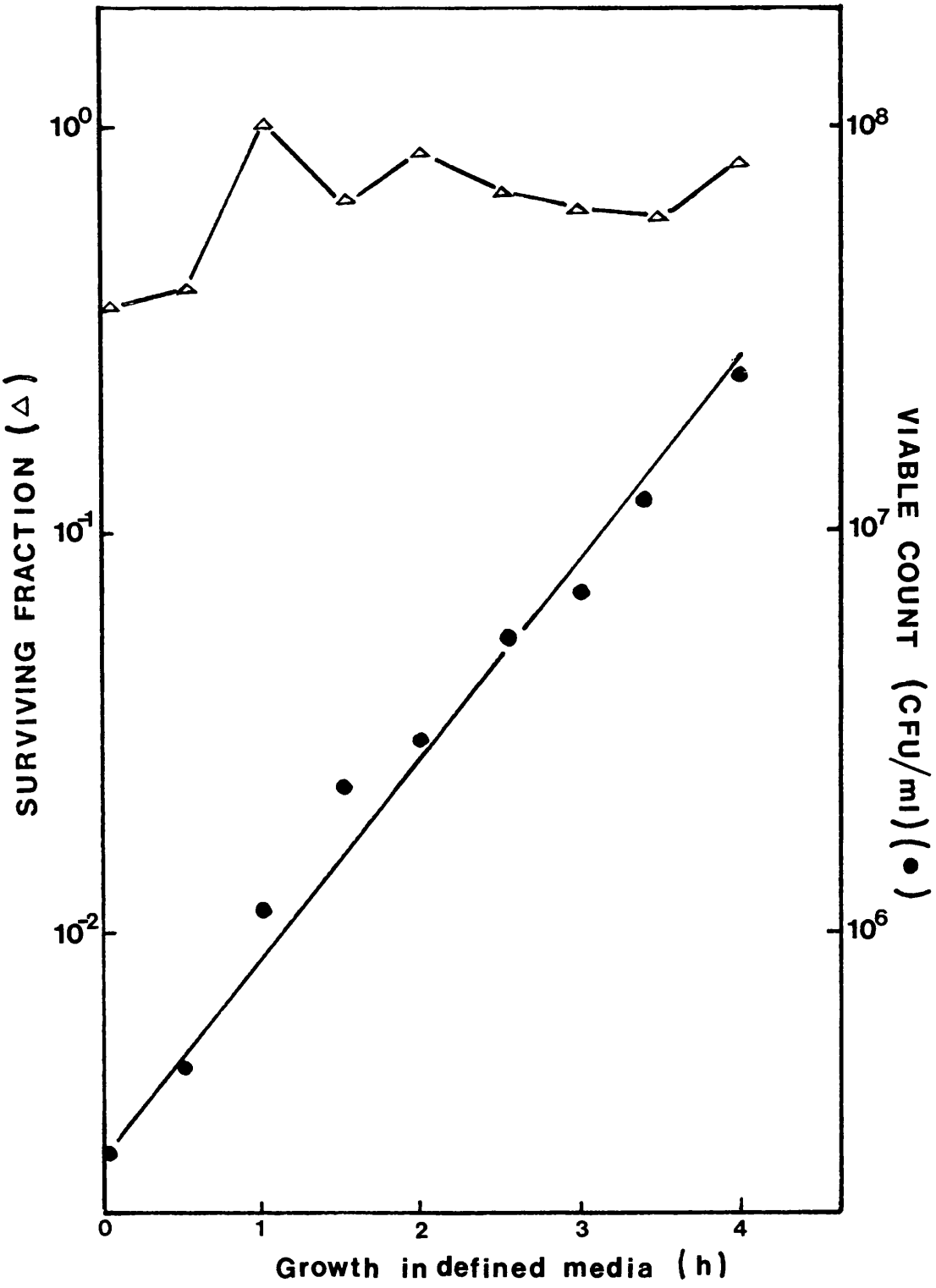


Figure 43: Surviving fractions (△) for *E. coli* B/r after near-UV irradiation for 60 min and viable counts (●) (CFU/ml) after growth in defined media for the number of hours shown on the abscissa: 2 h cells used for inoculum.

period studied. In addition, the initial dip in near-UV radiation sensitivity seen in figs. 38A, 39 and 42 has also been elminated, with survivial remaining at greater than 30% throughout. This suggests that the period of near-UV radiation sensitivity normally seen is not merely a product of subculturing the cells. It seems probably that the induced sensitivity is associated with lag phase since elimination of the lag phase eliminates the near-UV radiation sensitivity. Furthermore, fig. 44 shows that if the lag phase is not completely eliminated (by using cells grown for 1.5 h rather than 2 h as the inoculum) near-UV radiation sensitivity is observed but immediately after inoculation rather than after 2 h. This further suggests that it is the lag phase of growth that affects near-UV radiation sensitivity in *E. coli* B/r.

The Role of Stringent Control in Growth Phase Effects

The growth characteristics of bacterial cultures have been well defined for many years (Monod, 1949; Novick, 1955; Ingraham *et al.*, 1983). Broadly, three phases are observed; lag phase, exponential growth phase and stationary phase. Under ideal conditions, bacterial cultures grow in an unrestricted linear fashion (balanced growth) as seen in exponential phase. However, this state cannot continue as growing cells deplete nutrients in the growth media and produce waste products, which may accumulate and become toxic to the cells. When one or both of these events occur, cells pass through a period of unbalanced growth before entering stationary phase. When stationary phase cells are inoculated into fresh medium, exponential growth does not recommence immediately. Growth is delayed, and the length of this lag phase is dependent upon (a) the temperature, (b) the growth limiting factors in the original culture, (c) the concentration of cells in the stationary phase culture and (d) the length of time that cells

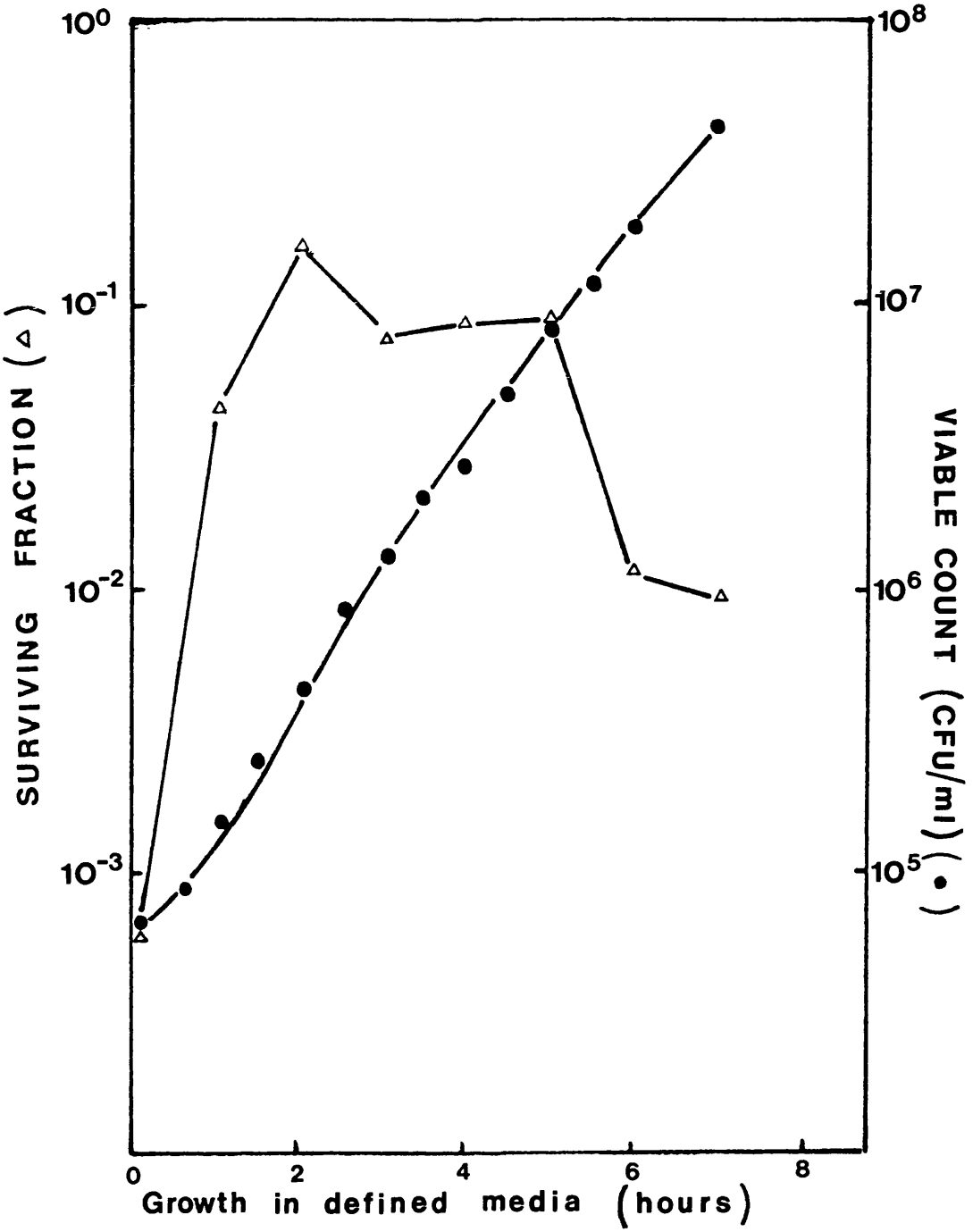


Figure 44: Surviving fractions (△) for *E. coli* B/r after near-UV irradiation for 60 min and viable counts (●) (CFU/ml) after growth in defined media for the number of hours shown on the abscissa: 1½ h cells used for inoculum.

were held in stationary phase (Ingraham *et al.*, 1983). Thus the nature of length of lag phase are dependent on the state of the cells in the previous stationary phase of growth.

Conditions such as amino acid starvation which may occur in the 24 h stationary phase cells used as the inoculum in the experiments represented in figs. 38 to 42, lead to the stringent response, as described in the general introduction. In this response, starved cells rapidly accumulate the nucleotide guanosine 3'-diphosphate 5'-diphosphate (ppGpp) (Cashel, 1969) which results in the shutoff of stable RNA synthesis and ultimately of protein synthesis. The stringent response has also been shown to have an important role in near-UV radiation effects (Ramabhadran, 1975; Ramabhadran and Jagger, 1976), with ppGpp being produced upon near-UV irradiation of cells. Normally this would lead to growth delay and other sublethal effects. However, it is possible that when cells are in lag phase, ppGpp levels are already raised in response to, for example, previous amino acid starvation of stationary phase cells and the additional effect of near-UV irradiation enhances the stringent effect. The cessation of protein synthesis would result in an inability to produce repair and protection enzymes which in turn would render cells susceptible to near-UV radiation damage.

Stringent control is not seen in relaxed (*rel*$^-$) strains under similar conditions to those that produce the response in their stringent (*rel*$^+$) counterparts. Thus, if the growth phase response seen in *E. coli* B/r was dependent on stringent control, this response would not be seen in *E. coli* B/r strains carrying the *relA*$^-$ mutation.

Therefore the growth phase response to near-UV radiation was studied in the isogenic *E. coli* B/r strains SR 833 (*relA*$^+$) and SR 834 (*relA*$^-$). The phenotype of these two strains was ascertained by growth delay experiments the results of which are shown in fig. 45. Exponentially growing cells were

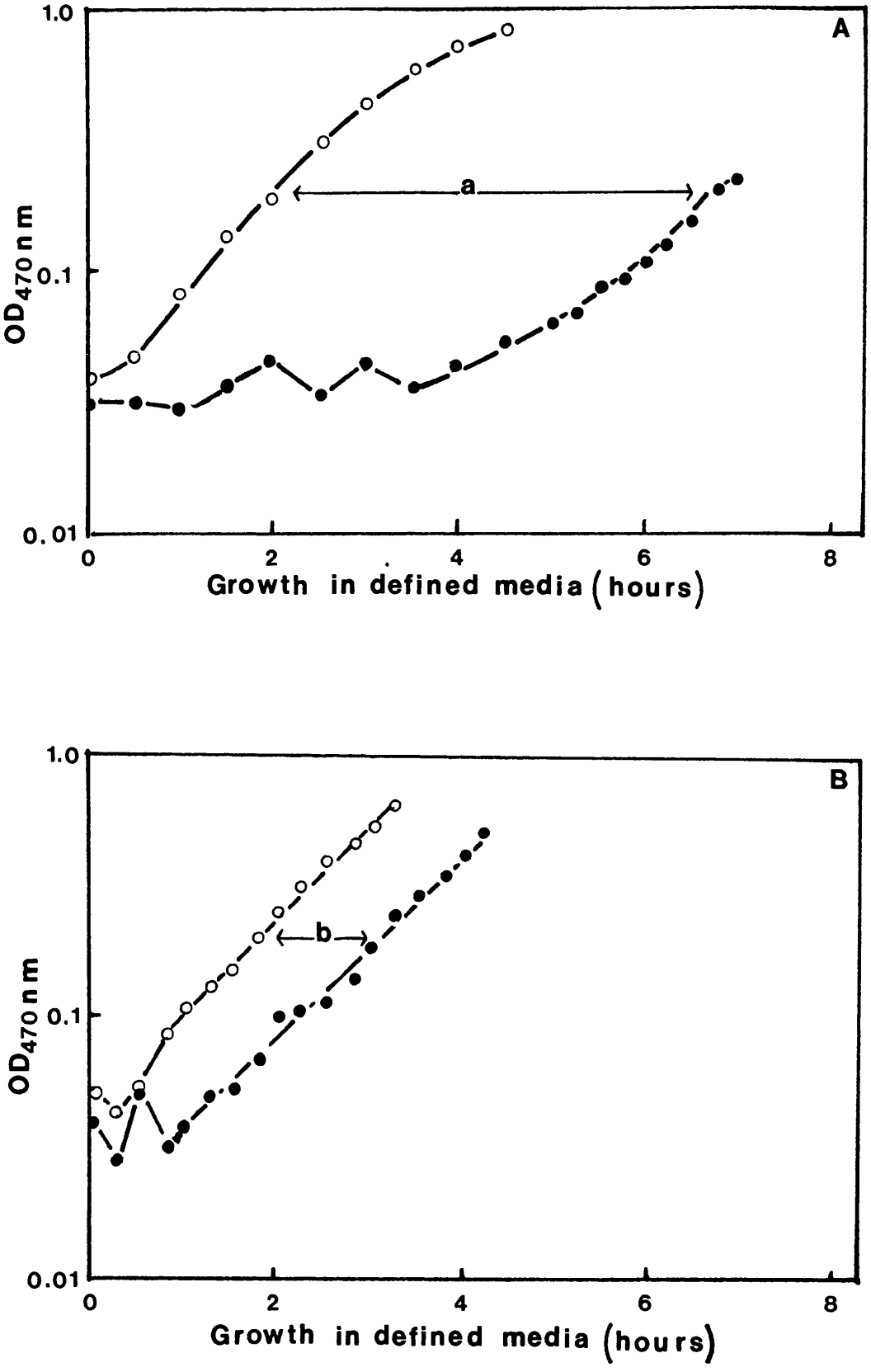


Figure 45: Growth curves for (A) SR833 *relA*$^+$ and (B) SR834 *relA*$^-$; growth of unirradiated controls (o) and irradiated samples (●) in defined media for the number of hours shown on the abscissa: a and b = near-UV radiation induced growth delay.

harvested by membrane filtration. Half of the cell sample was irradiated with broad-band near-UV radiation for 15 min before being reinoculated into fresh, prewarmed media, whilst the remaining cells were used as an unirradiated control. The response of the *relA*$^{+}$ strain SR 833 is seen in fig. 45A. With the unirradiated control, exponential growth followed an initial lag of approximately 0.5 h. The irradiated sample showed a greatly extended lag period before growth resumed, giving a growth delay (a) of approximately 4.5 h. With the relaxed *relA*$^{-}$ strain, SR 834 (fig. 45B), the unirradiated control showed a similar growth pattern to SR 833, but the growth delay (b) shown by the irradiated sample was only approximately 1.5 h.

Survival curves for exponential phase cells of both SR 833 and SR 843 are shown in fig. 46. SR 833 (*relA*$^{+}$) was shown to be more sensitive to near-UV radiation than SR 834 (*relA*$^{-}$), the former showing 3½ log kill after 80 min irradiation, the latter giving approximately 1 log kill for the same length of irradiation. The effect of phase of growth on near-UV radiation sensitivity of these two strains was then studied. Survival after 60 min near-UV irradiation was followed for growth over an 8 h period. The results for both strains are shown in fig. 47. The result seen with SR 833 (*relA*$^{+}$) is very similar to that seen with B/r (fig. 38A). Cells were initially resistant to near-UV radiation. This was followed by a rapid increase in sensitivity during exponential phase with a maximum at 3 h, followed by a return to the original degree of resistance by the end of exponential phase. With the relaxed strain SR 834 (*relA*$^{-}$), the effect of near-UV radiation was much less marked. A slight increase in sensitivity was seen after 3 h growth but this was considerably smaller than the corresponding increase in SR 833. This suggests that the stringent response is indeed important in the growth phase response of *E. coli* B/r strains to near-UV irradiation.

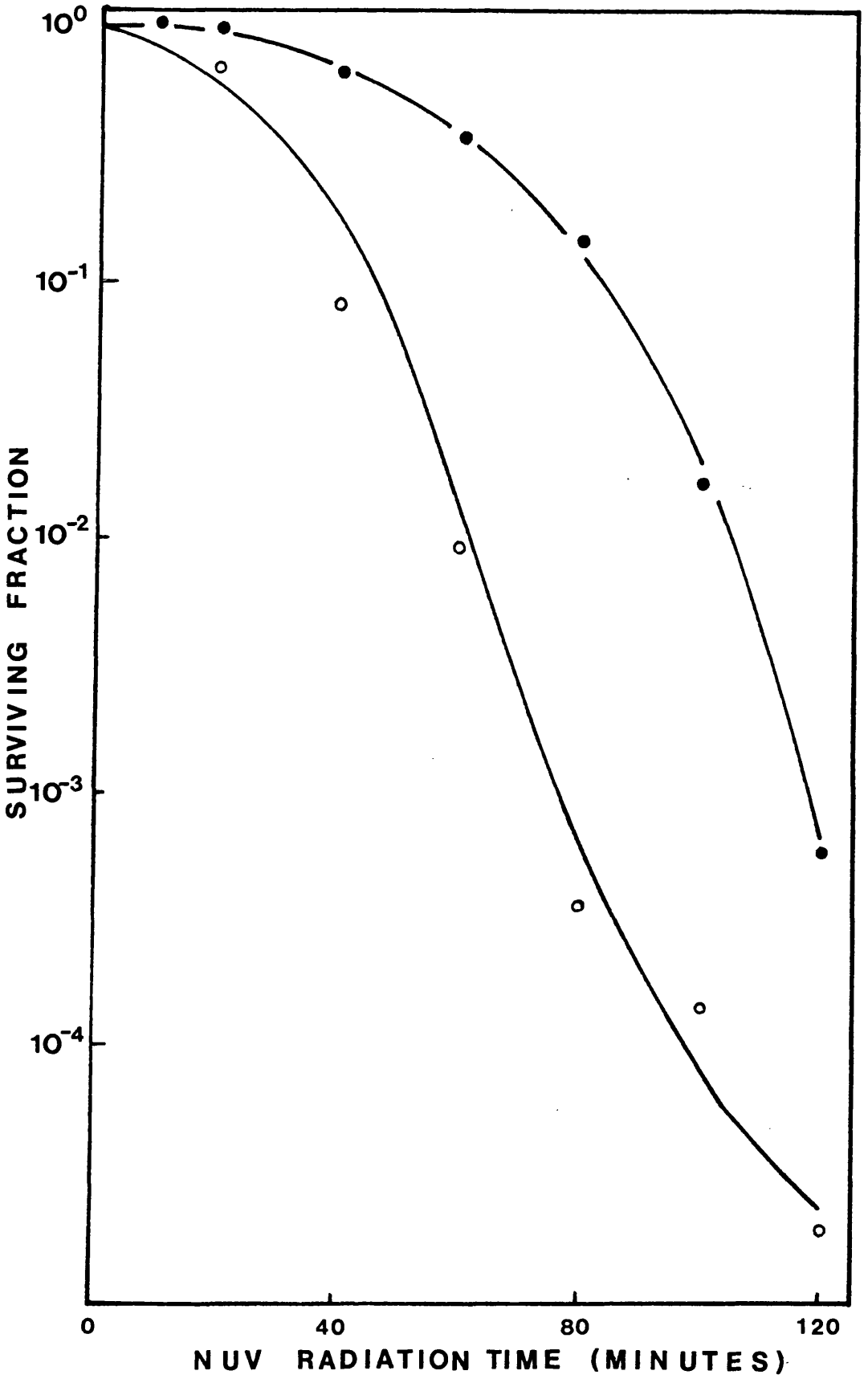


Figure 46: Survival curves for *E. coli* SR833 *relA*$^+$ (o) and *E. coli* SR834 *relA*$^-$ (●) after near-UV irradiation of 3 h exponential phase cells.

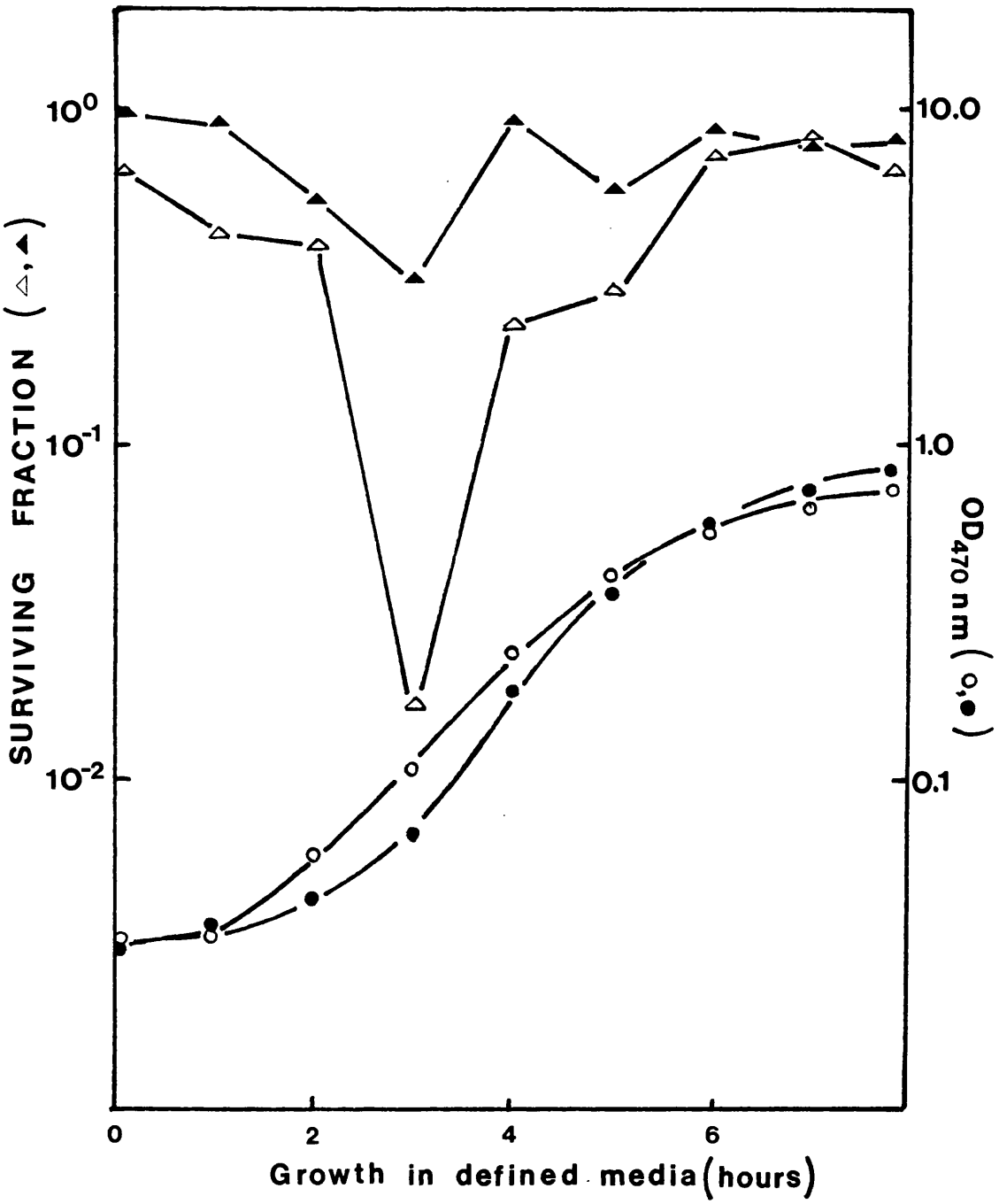


Figure 47: Surviving fractions (△,▲) after 60 min near-UV irradiation and optical densities (○,●) at 470 nm for *E. coli* SR833 *relA*$^+$ (△,○) and *E. coli* SR834 *relA*$^-$ (▲,●) after growth in defined media for the number of hours shown on the abscissa.

Results presented in figs. 36-40 showed that the growth phase response of *E. coli* K12 AB1157 was considerably different from that of *E. coli* B/r. One reported difference between *E. coli* K12 strains and *E. coli* B/r strains is that greater levels of ppGpp accumulate in B/r strains after the near-UV irradiation of exponentially growing cells than accumulate in K12 strains (Thiam and Favre, 1984). If, as has been suggested above, stringent control is important in growth phase effects, then the strain dependency of ppGpp accumulation may be related to the differences seen with B/r and AB1157 in response to near-UV radiation. To examine this, the experiments carried out with SR 833 and SR 834, both B/r strains, were repeated using an isogenic *relA*$^+$/*relA*$^-$ pair of *E. coli* K12 strains, NF 161 (*relA*$^+$) and NF 162 (*relA*$^-$). Survival curves were obtained after irradiation of cells in early exponential phase (3 h), late exponential phase (6 h) and stationary phase (24 h) and the results are shown in fig. 48. With NF 161 (*relA*$^+$: fig. 48A) greatest sensitivity to near-UV radiation was seen with 3 h cells, an intermediate sensitivity was seen with 6 h cells and stationary phase cells (24 h) were very resistant. This response was more like that of B/r (fig. 37A) than of 1157 (fig. 37B). Survival curves of NF 162 (*relA*$^-$: fig. 48B) show 6 h cells to be most sensitive whilst both 3 h and 24 h cells show a similar, more resistant response.

The growth phase response of these two strains was then followed in a single experiment over a 10 h period. The results for both strains are shown in fig. 49. These results confirm that the response of NF 161 (*relA*$^+$) is very similar to the growth phase response of B/r (fig. 38A), rather than that of AB1157 (fig. 38B). NF 161 cells were initially resistant to near-UV radiation. This was followed by a large increase in sensitivity that reached a maximum after approximately 5 h growth in defined media. Unlike the response of B/r (fig. 38A) this increase in sensitivity appeared to occur during mid-exponential phase rather than at the end of lag phase. Sensitivity

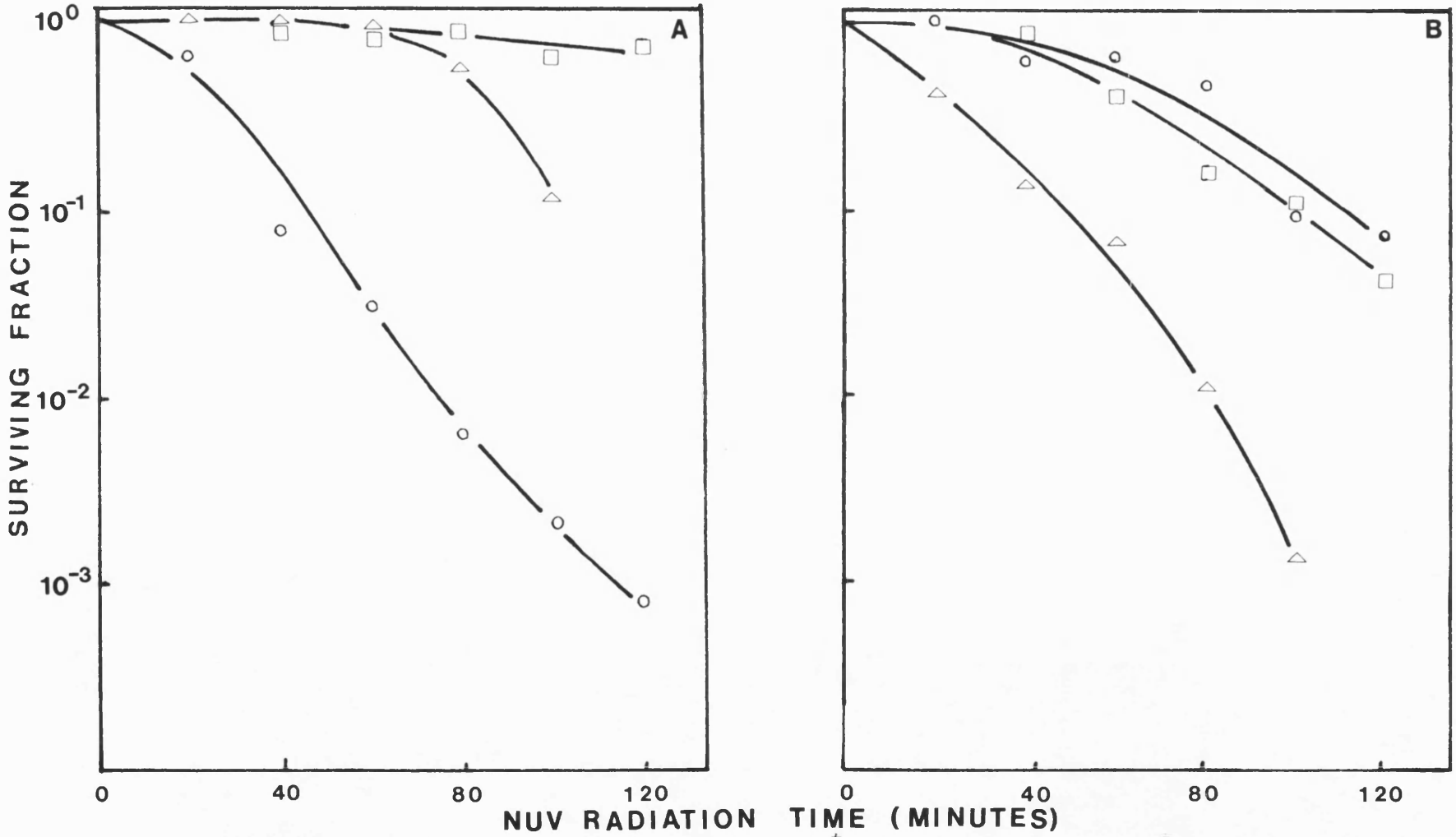


Figure 48: Survival curves for (A) *E. coli* NF161 *relA*$^+$ and (B) *E. coli* NF162 *relA*$^-$ after growth in defined media for 3 h (o), 6 h (△) and 24 h (□).

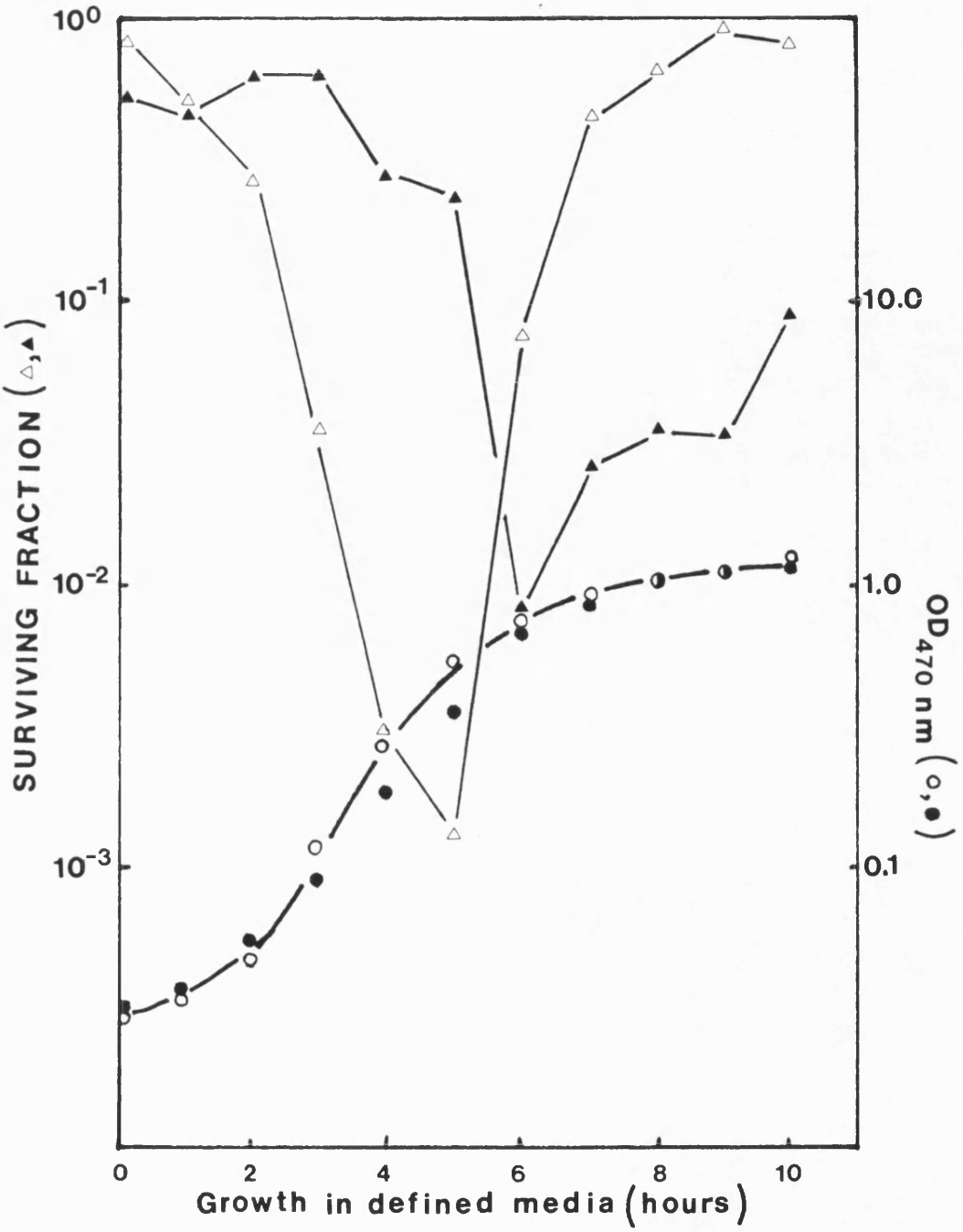


Figure 49: Surviving fractions (△ , ▲) after 90 min near-UV irradiation and optical densities (○ , ●) at 470 nm for *E. coli* NF161 *relA*$^-$ (△ , ○) and *E. coli* NF162 *relA*$^+$ (▲ , ●) after growth in defined media for the number of hours shown on the abscissa.

decreased throughout the remainder of exponential phase, returning to the initial level by the end of exponential phase.

The response of NF 162 (*relA*$^-$) exhibited a different pattern to that shown by NF 161 (*relA*$^+$). Cells were more resistant to near-UV irradiation for the first 5 h of growth of the culture, the period of increasing sensitivity for NF 161. This was then followed by a rapid increase in sensitivity, although this was not as great as that shown at 5 h by NF 161. Throughout the remainder of the experiment, the period equivalent to late exponential phase, near-UV sensitivity slowly decreased, although by the end of the period studied, cells were still considerably more sensitive to near-UV radiation than seen initially.

The results presented in fig. 49 show that for these two K12 strains, the growth phase response to near-UV radiation is affected by the *relA*$^+$/*relA*$^-$ phenotype. However, the response exhibited by NF 161 (*relA*$^+$) is very different to that shown by the other K12 *relA*$^+$ strain used, AB1157 (fig. 38B).

Discussion

The results presented in this section illustrate the importance of the phase of growth of cells when studying near-UV radiation effects. In all of the strains studied (with the exception of SR 834, fig. 47) large variations in sensitivity to near-UV radiation were seen at different phases of growth. For example, with *E. coli* B/r (figs. 38A and 39) a difference of 3 log cycles of inactivation was seen between stationary phase cells and cells grown for 2 h in defined media. The variations in near-UV radiation sensitivity were most marked in exponential phase (again with the exception of SR 834, fig. 47, where no great variation in near-UV radiation sensitivity was seen over the 8 h period of growth studied). Peak (1970) presented data for *E. coli* B/r that is in agreement with fig. 38A and suggested that during rapid growth,

cells are unable to efficiently recover from near-UV radiation damage. Although this could explain the sensitivity of B/r in early exponential phase, the response of K12 AB1157 (fig. 38B) cannot be accounted for by this explanation. Peak (1970) also showed that growth phase effects are considerably less important after far-UV radiation of *E. coli* B/r than after near-UV radiation, although growth phase variations have been reported in *E. coli* strains that differ in repair characteristics after 254 nm irradiation (Tyrrell *et al.*, 1972).

The differences seen in near-UV radiation sensitivity with varying growth phase may reflect different mechanisms and degrees of inactivation and repair between stationary and exponential phase cells. Mechanisms that are important in one phase of growth may be less important or overshadowed by different mechanisms in other phases of growth. A fuller discussion of this was included in the general introduction.

The results presented in figs. 38B and 40 show that the response of *E. coli* K12 AB1157 was the opposite of that seen with *E. coli* B/r (figs. 38A and 39). This has also been reported by Kelland *et al.*, (1984b) for *E. coli* K12 AB1157 and another K12 derivative *E. coli* SR 385. These differences seen in the growth phase responses of different strains indicate that near-UV radiation sensitivity is strain dependent. This may imply that different mechanisms and possibly different targets and chromophores are important in strains of different origin. Near-UV radiation related differences between B/r and K12 have been reported. Firstly, K12 strains appear to have a second minor chromophore for near-UV radiation induced growth delay, other than ^{4}Srd, which is not seen in *E. coli* B strains. This has been identified as 2-thiouridine (Favre and Thomas, 1981). Secondly, K12 requires much higher fluences of near-UV

radiation than B/r to reduce the capacity of the bacteria to support phage growth (reviewed in greater detail by Jagger, 1985).

The series of experiments carried out on B/r (figs. 41-44) established that the period of high sensitivity seen in fig. 38A was associated with the end of lag phase or early exponential phase growth. Extending the exponential phase of growth did not increase the period of sensitivity (fig. 42) and eliminating the lag phase eliminated the period of sensitivity (fig. 43). The growth phase data presented by Peak (1970) also shows that the rapid increase in near-UV sensitivity seen with B/r occured at the end of lag phase after an initial period of high resistance.

The results presented in figs. 45 to 49 suggest that stringent control has a role to play in growth phase effects in *Ecoli* strains. Two pairs of isogenic stringent/relaxed strains were studied and in both cases that *relA*$^-$ (relaxed) strains showed an altered growth phase response as compared to their *relA*$^+$ (stringent) counterparts (figs. 47 and 49). In fig. 47, two B/r strains SR 833 and SR 834 were studied. The *relA*$^+$ response was very similar to the original B/r response seen in fig. 38A, but with the *relA*$^-$ strain the large increase in sensitivity seen in early exponential or late lag phase was eliminated. These results would seem to support the suggestion, discussed earlier, that lag phase cells may 'carry over' certain characteristics of the stationary phase cells used to inoculate the new culture. However, it is also possible that the increased sensitivity seen in B/r after 2 h growth may be directly related to the state of cells at the end of lag phase and not indirectly to a previous growth phase.

The results obtained with the corresponding K12 strains, NF 161 and NF 162 are shown in figs. 48 and 49. With the K12 related *relA*$^+$ strain NF 161 (figs 48 and 49) the results did not correlate with those seen with K12 AB1157 (fig. 38B). However, although the response of the *relA*$^+$ strain NF 161 was

completely different from K12 AB1157, there were similarities between the *relA*$^-$ strain NF 162 and AB1157. With both of these strains there was increased sensitivity in mid to late exponential phase, although with NF 162 this was followed by decreasing sensitivity which was not seen with AB1157.

When the results obtained with NF161 and NF 162 are compared to the B/r strains, it is seen that the response of NF 161 was similar to that of B/r (fig. 38A) and SR 833 (fig. 47). However, the response of the relaxed strain NF 162 was different from the relaxed B/r strain SR 834 (figs. 49 and 47 respectively). SR 834 (fig. 47) remained relatively resistant to near-UV radiation throughout the 8 h period studied whilst with NF 162 (fig. 49) there was a considerable increase in sensitivity after 5 h growth in defined media. Thus the response of NF 161 and NF 162 was different from both the B/r strains and K12 AB1157.

The differences seen between K12 AB1157 and the isogenic *relA*$^+$/*relA*$^-$ K12 pair may be partially explained by a fundamental phenotypic difference between the strains which may have important consequences on growth phase effects if stringent control is involved. NF 161 and NF 162 carry the *spoT1* mutation. *spoT* mutants show greatly reduced turnover of ppGpp (Laffler and Gallant, 1974) resulting in high levels of ppGpp, two to three times higher than in *spoT*$^+$ cells. In this respect NF 161 and NF 162 may be similar to B/r since Thiam and Favre (1984) have reported that higher levels of ppGpp accumulate in B/r *relA*$^+$ than in K12 *relA*$^+$ strains. Thus NF 162, which is *spoT*$^-$, *relA*$^-$ may have raised levels of ppGpp as compared to a purely *relA*$^-$ strain. Indeed, it has been reported that in certain leaky *relA*$^-$ mutants carrying *spoT*$^-$, the reduced turnover of ppGpp has led to accumulation of ppGpp to near stringent levels which has resulted in suppression of the *rel*$^-$ phenotype (Fiil *et al.*, 1977). It would be

interesting and probably informative to study the growth phase responses of K12 *rel*$^{+}$/*rel*$^{-}$ strains that did not carry the mutation in *spoT*.

RESULTS: SECTION 3

Investigations into the Near-Ultraviolet Radiation Sensitivity of *Escherichia coli* SR246

PART I: Characterization of the near-UV radiation sensitivity of SR246.

Materials

1) Media

Cultures were grown in defined media, nutrient broth or yeast extract nutrient broth (YENB). Defined media for SR246 and nutrient broth were described in the general methodology. YENB was made by dissolving nutrient broth (8g, Difco) and yeast extract (7.5g, Difco) in 1 l distilled water. This was sterilized as 100 ml aliquots in 150 ml screw top bottles by autoclaving at 121°C for 20 min.

YENB agar plates (described in the general methodology) were used routinely in this section unless otherwise stated.

2) Rose Bengal

Rose bengal impregnated polypropylene balls (Sensistox, Hydron Laboratories Inc., New Brunswick; kindly donated by Dr T. Ito, Tokyo, Japan) were sterilized in M9 salts solution (14 mg/ml) by autoclaving at 121°C for 20 min. Since the rose bengal polypropylene balls settle out of suspension very quickly, it was necessary to mix the suspension thoroughly by vortexing before each manipulative procedure.

Methods

Exposure to 548 nm Light in the Presence of Rose Bengal

Cells were grown to stationary phase and harvested as described in the general methodology. Cells were diluted to 2 x 10^7 CFU/ml in M9 salts solution and 1.5 ml added to 1.5 ml 14 mg/ml rose bengal suspended in M9 salts solution, giving final concentrations of 10^7 CFU/ml in 7 mg/ml rose

bengal suspension. Cell suspensions were prepared immediately before the start of an experiment. Irradiation was with 548 nm light. This was produced from a Bausch and Lomb high intensity grating monochromator used in conjunction with a Bausch and Lomb SP200 super pressure mercury lamp (as described in the general methodology) fitted with a Corning 0.51 stray-light filter and at a fluence rate of 73.6 $Jm^{-2}s^{-1}$. Samples were diluted in M9 salts solution, plated on YENB agar and incubated at 37°C for 48 h. The irradiation procedure and determination of fluence rate are described in the general methodology. The cell suspension was stirred rapidly throughout irradiation to prevent the rose bengal beads from settling out.

Results

Effects of Pre- and Post-Irradiation Growth Media

Kelland *et al.* (1983 a and b) have shown that the composition of the post-irradiation plating media can play an important part in the assessment of viability of *E. coli* cells after exposure to near-UV radiation. To examine this, SR246 was initially grown in defined media and survival after near-UV irradiation was assessed on defined media plates, YENB agar and tryptone agar. The results of this experiment are shown in fig. 50. The survival curves for defined media-grown, stationary phase SR246 plated on both YENB agar and tryptone agar exhibit no kill over the first 40 min of irradiation whereas the cells plated on defined media show little or no shoulder region over the same period on the survival curve. All three curves show 2 to 3 log kill over 120 min near-UV irradiation. This result does not indicate that SR246 is particularly sensitive to near-UV radiation when compared to, for example, stationary phase *E. coli* K12 AB1157 (fig. 34A) as had been previously reported. Since it was known that previous work had been carried out using stationary phase cells grown in

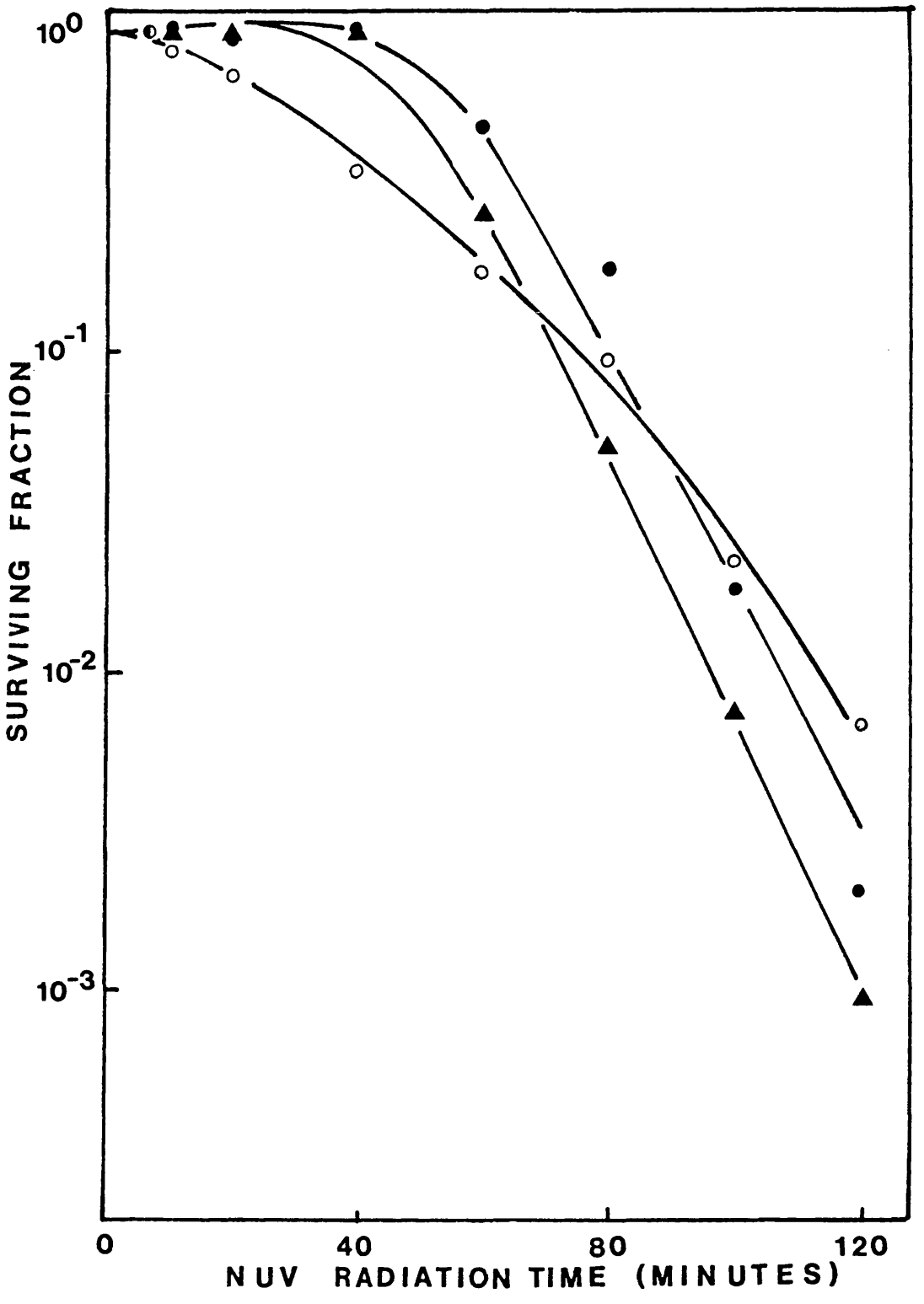


Figure 50: Survival curves for *E. coli* SR246 after near-UV irradiation. Pre-irradiation growth was in defined media and survival was assessed on YENB agar plates (▲), tryptone agar plates (●) and defined media agar (o).

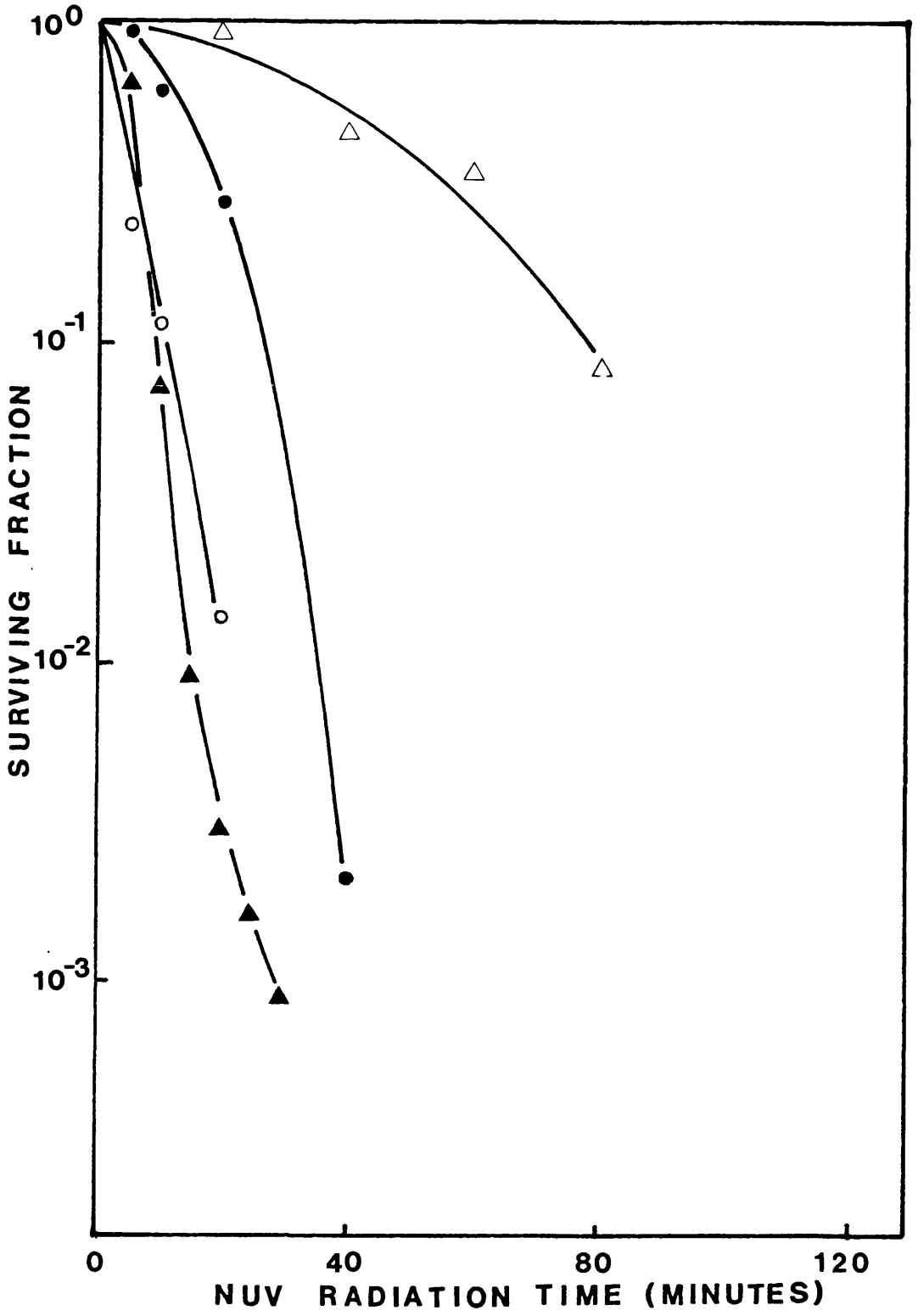


Figure 51: Survival curves for *E. coli* SR246 after near-UV irradiation. Pre-irradiation growth was in YENB and survival was assessed on YENB agar plates (▲), tryptone agar plates (●) and defined media agar (o). The response of *E. coli* SR385 grown in YENB, irradiated and assessed on YENB agar is also shown (△).

rich media, the above experiment was repeated using YENB as the pre-irradiation growth media. The results shown in fig. 51 show a very marked increase in near-UV radiation sensitivity when compared to fig. 50. Also shown in fig. 51 is the survival of stationary phase SR385 after near-UV irradaition, for cells grown in YENB and plated on YENB agar. This is included as a comparative control to show the response of a non-sensitive strain, grown under the same conditions that lead to near-UV radiation sensitivity in SR246. In order to ascertain whether or not the difference between YENB-grown and defined media-grown cells was characteristic of YENB or of other rich media as well, SR246 was also grown in nutrient broth (Oxoid) and, after near-UV irradiation, plated on nutrient agar plates. The response of cells grown in nutrient broth and plated on nutrient agar (from a single experiment, fig. 52) is similar to the result shown in fig. 51. However, there is a difference between cells grown in defined media and plated on nutrient agar (fig. 52) and the results seen in fig. 50 where cells grown in defined media were plated on defined agar, YENB agar or tryptone agar. Cells plated on nutrient agar are considerably more sensitive to near-UV radiation than cells plated on any of the other three plate types. Moss and Smith (1981) and Kelland *et al.* (1983b) have reported a sensitivity to inorganic salt after irradiation with broad-band near-UV radiation. Kelland (1984, PhD thesis) showed that near-UV radiation survival of repair competant stationary phase *E. coli* SR385 grown in defined media was variable and dependent on the plating media used to assess viability. Survival was lowest when cells were plated on Oxoid nutrient agar and a more resistant response was obtained when cells were plated on defined media agar or YENB agar. This was found to be due to the high salt content of Oxoid nutrient agar and occurred with near-UV but not far-UV radiations. It may be that this near-UV radiation induced salt sensitivity is the reason behind the sensitivity of defined media-grown

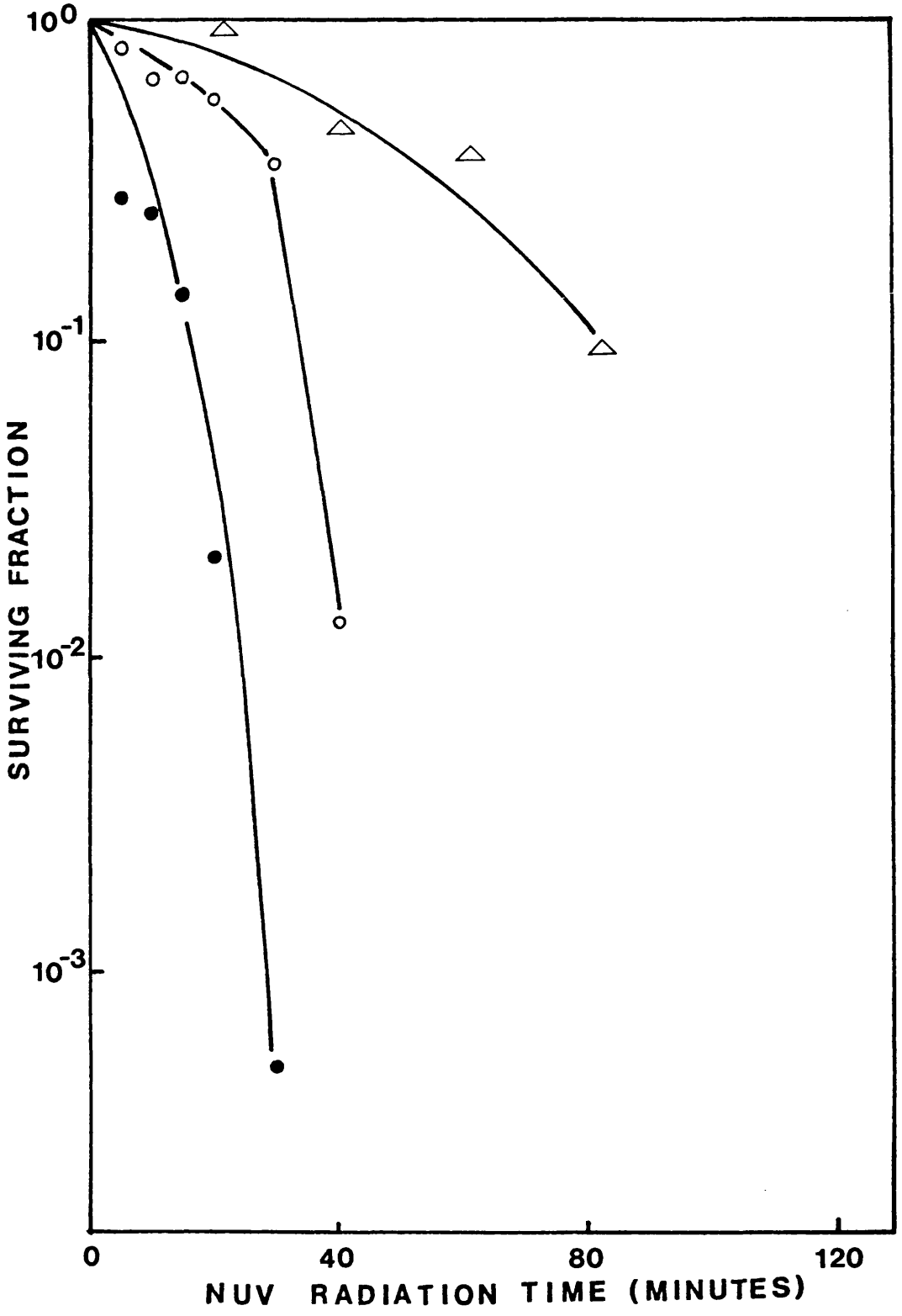


Figure 52: Survival curves for *E. coli* SR246 after near-UV irradiation. Pre-irradiation growth was in nutrient broth (●) or defined media (o) and survival was assessed on nutrient agar (oxoid). The response of *E. coli* SR385 grown in YENB, irradiated and assessed on YENB agar is also shown (△).

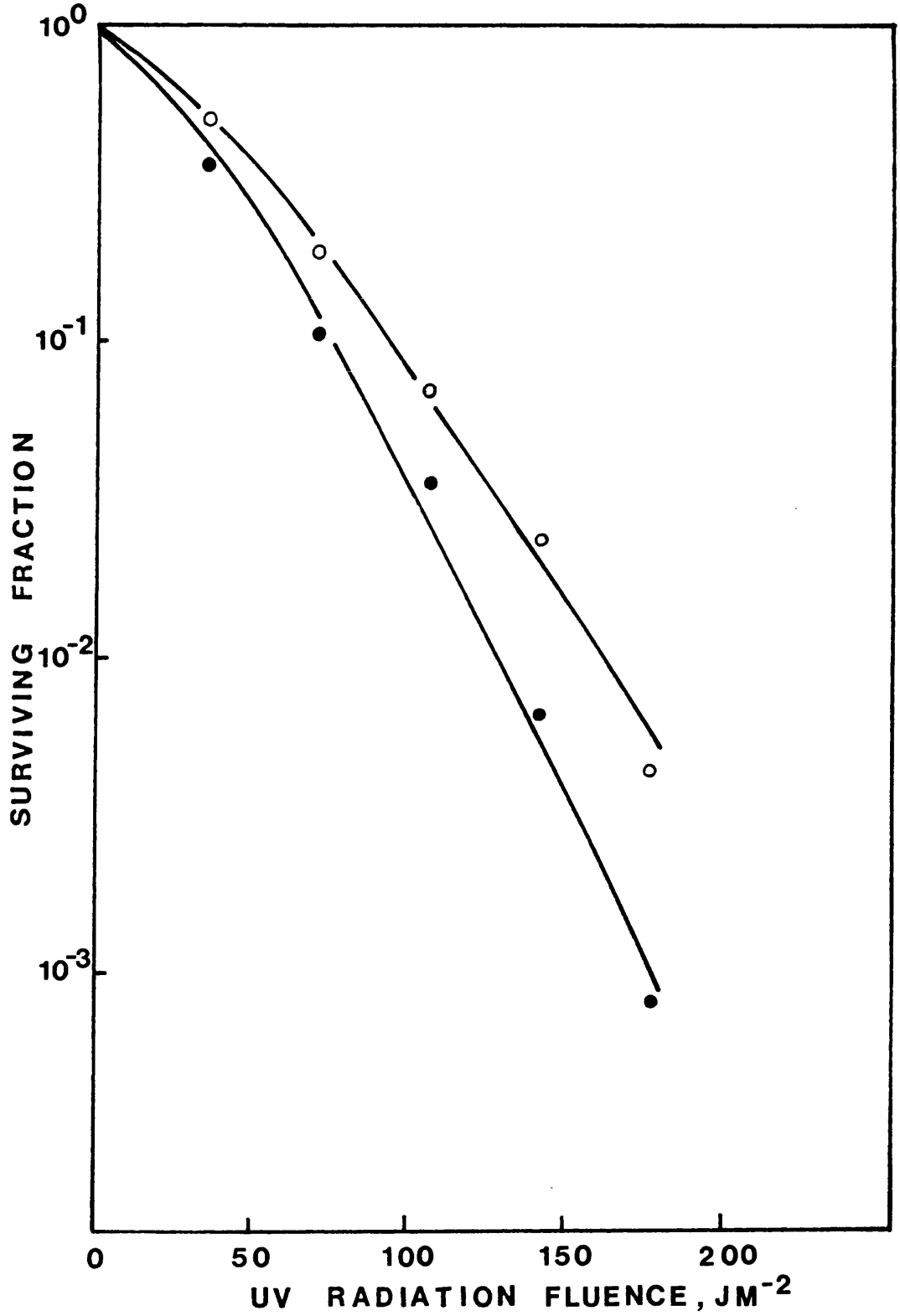


Figure 53: Survival curves for *E. coli* SR246 after 254 nm irradiation. Pre-irradiation growth was in YENB (●) or defined media (○) and survival was assessed on YENB agar plates.

SR246 seen in fig. 52. It may also be that this near-UV radiation induced salt sensitivity is only important for cells grown in defined media, since the cells grown in nutrient broth were no more sensitive than cells grown in YENB, regardless of the post-irradiation plating medium.

SR246 was also irradiated with 254 nm radiation after growth in YENB or defined media (fig. 53). Although there is a significant difference between the two responses, with cells grown in YENB being more sensitive to far-UV radiation than cells grown in defined media, the effect is considerably less marked than that seen after near-UV irradiation. This would indicate that the radiation sensitivity of SR246 seen under certain growth conditions is primarily a near-UV radiation effect.

Effect of Oxidative Damage on SR246

Near-UV radiation induced damage involves the production of active oxygen species, amongst which singlet oxygen ($^1O_2$) may have an important role (Tuveson, 1980; Peak *et al.*, 1981; reviewed Webb, 1977; Jagger, 1985). Thus, studying the effect of singlet oxygen may give an insight as to how near-UV radiation causes damage. In a single experiment SR246 was challenged with singlet oxygen which, in this case, was produced by 548 nm irradiation of a cell suspension containing rose bengal impregnated polypropylene balls. SR246 was found to be sensitive to single oxygen when compared to an Hfr-Tn10 strain, BW6163 and to the SR246 x BW6163 conjugant, X14/50, both of which are near-UV radiation resistant, fig. 54 (BW6163 and X14/50 will be described in full later in this section of results). A difference in sensitivity to singlet oxygen was also seen, in a single experiment, between SR246 grown in defined media and SR246 grown in nutrient broth (fig. 55). Cells grown in nutrient broth and plated on YENB were more sensitive to singlet oxygen than cells grown in defined

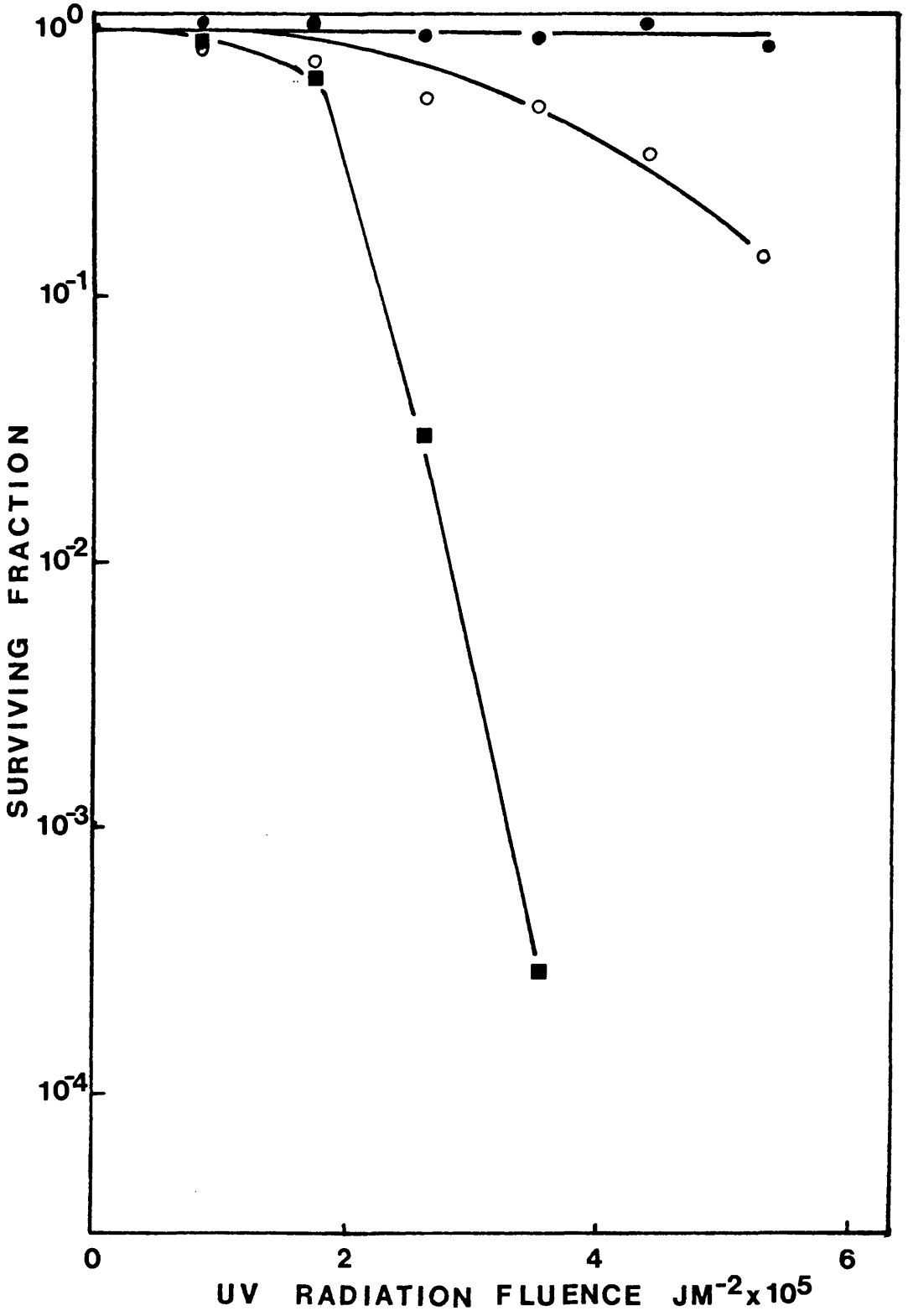


Figure 54: Survival curves for *E. coli* SR246 (■), *E. coli* BW6163 (o) and *E. coli* X14/50 (●) after irradiation at 548 nm in the presence of rose bengal with viability assessed on YENB plates.

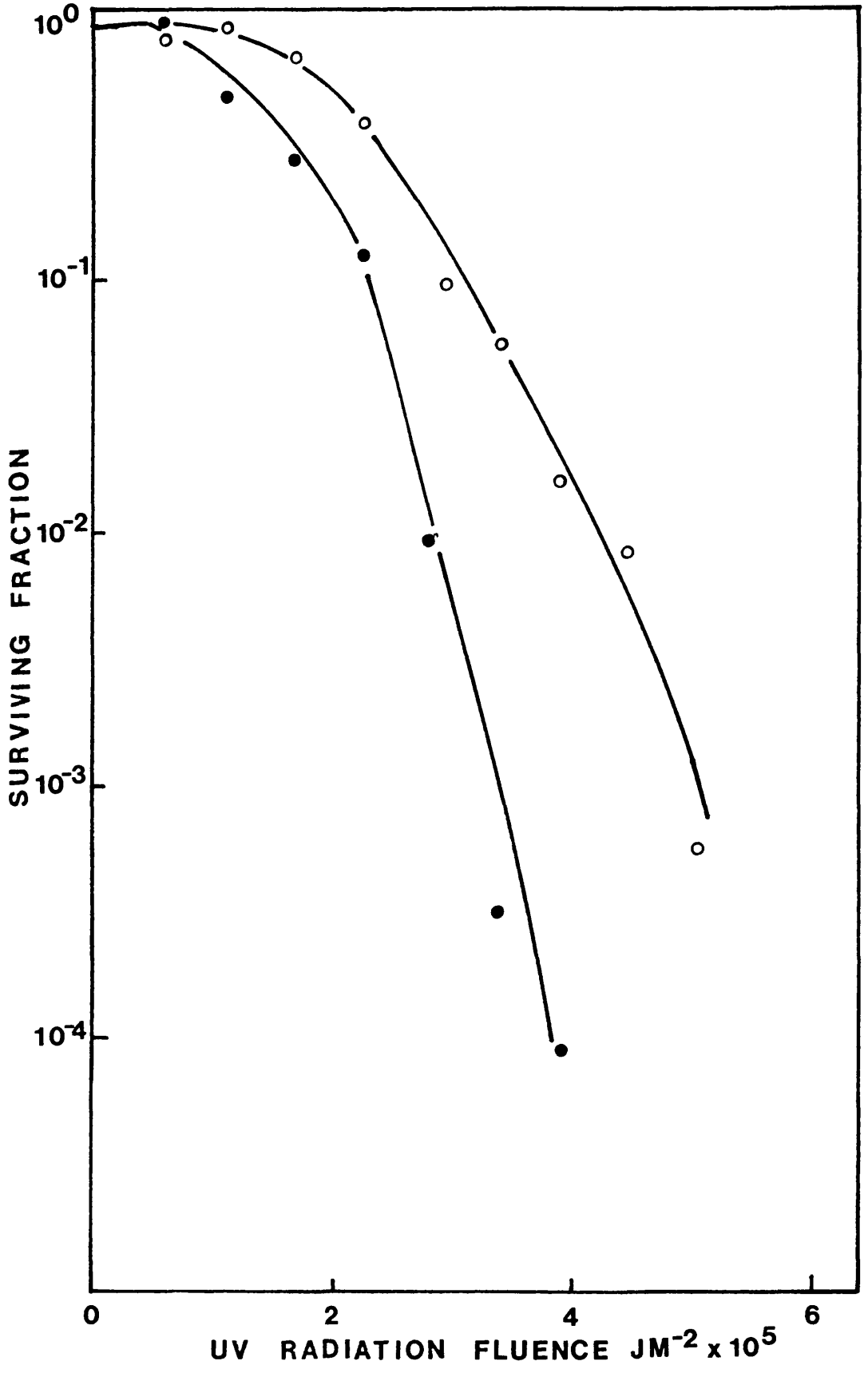


Figure 55: Survival curves for *E. coli* SR246 after irradiation at 548 nm in the presence of rose bengal with cells grown in nutrient broth (●) or defined media (○) with viability assessed on YENB plates.

media and plated on YENB. This is the same response as that seen for near-UV radiation sensitivity after growth in rich or defined media, although the difference in sensitivity was less marked in this one experiment for the singlet oxygen exposure than for near-UV irradiation.

As well as the production of active oxygen species being implicated in near-UV radiation induced damage, it has also been shown that near-UV irradiation of tryptophan results in the production of H_2O_2 (McCormick *et al.*, 1976), and that peroxides also give rise to damaging oxygen species (Haber and Weiss, 1934). Thus it is possible that H_2O_2 and near-UV radiation kill at least in part, by similar mechanisms. The role of H_2O_2 in near-UV radiation damage and the relevant literature were discussed and reviewed in the general introduction and Section 1 of the results. If H_2O_2 is produced during near-UV irradiation and this in turn produces singlet oxygen, to which SR246 is sensitive, it would be expected that the presence of catalase, which degrades H_2O_2, would decrease the sensitivity of SR246 to near-UV radiation. Catalase incorportated into the plating media was found to protect SR246 and increased survival from 4 log kill to 1 log kill after 30 min near-UV irradiation to a level approaching that of SR385, plated on YENB without catalase, fig. 56. Heat inactivated catalase afforded no protection. SR246 did not appear to be deficient in catalase activity itself as the assay for H_2O_2 scavenging ability, which is mainly due to catalase, shows that SR246 is efficient in degrading H_2O_2 when compared to the catalase deficient mutant, UMI (fig. 57), showing a similar H_2O_2 scavenging ability as that of SR385 when treated under the same conditions.

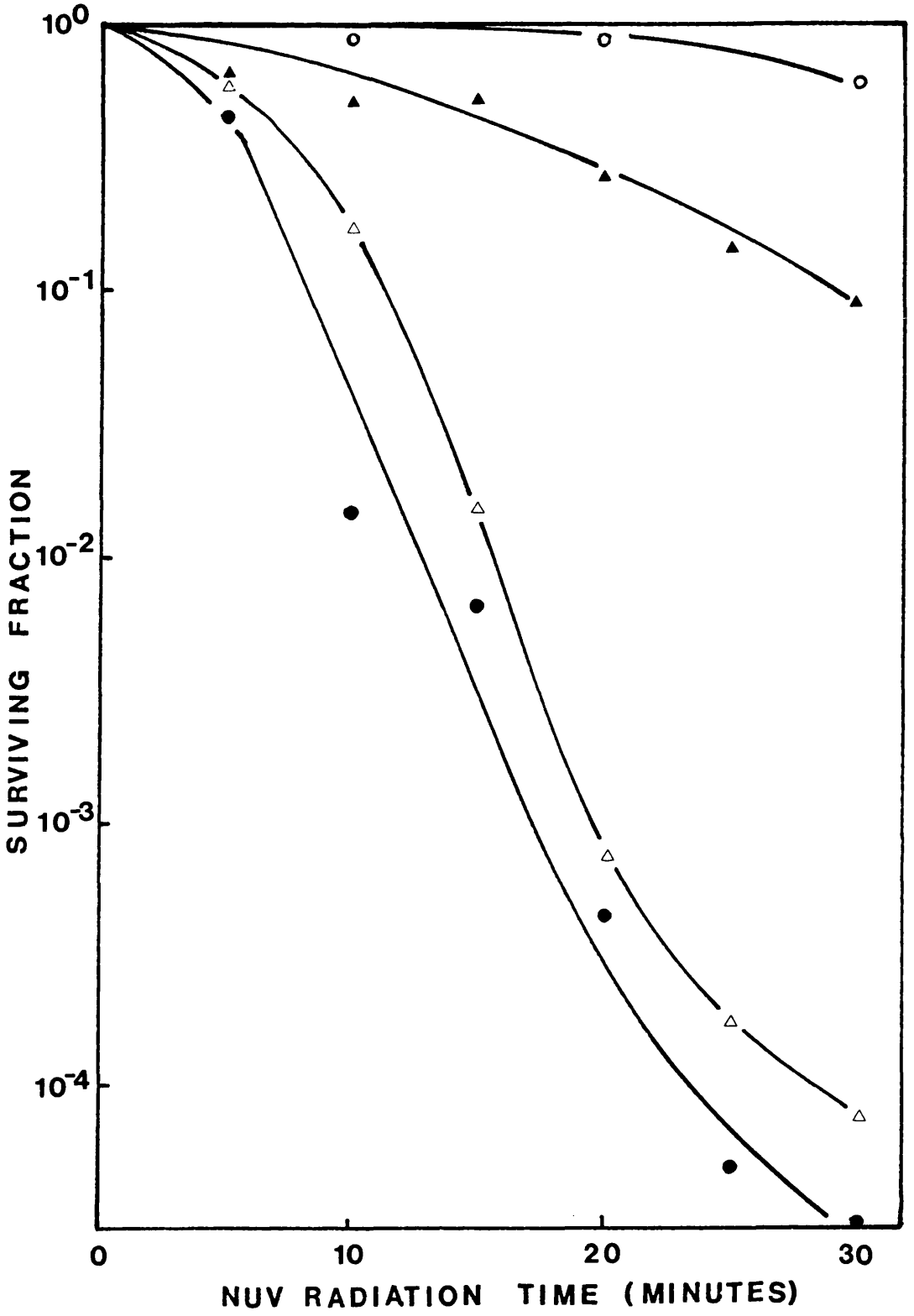


Figure 56: Survival curves for *E. coli* SR246 after near-UV irradiation with viability assessed on YENB (△), YENB + catalase (▲) and YENB + heat-inactivated catalase (●). The response of *E. coli* SR385 grown in YENB, irradiated and assessed on YENB is also shown (o).

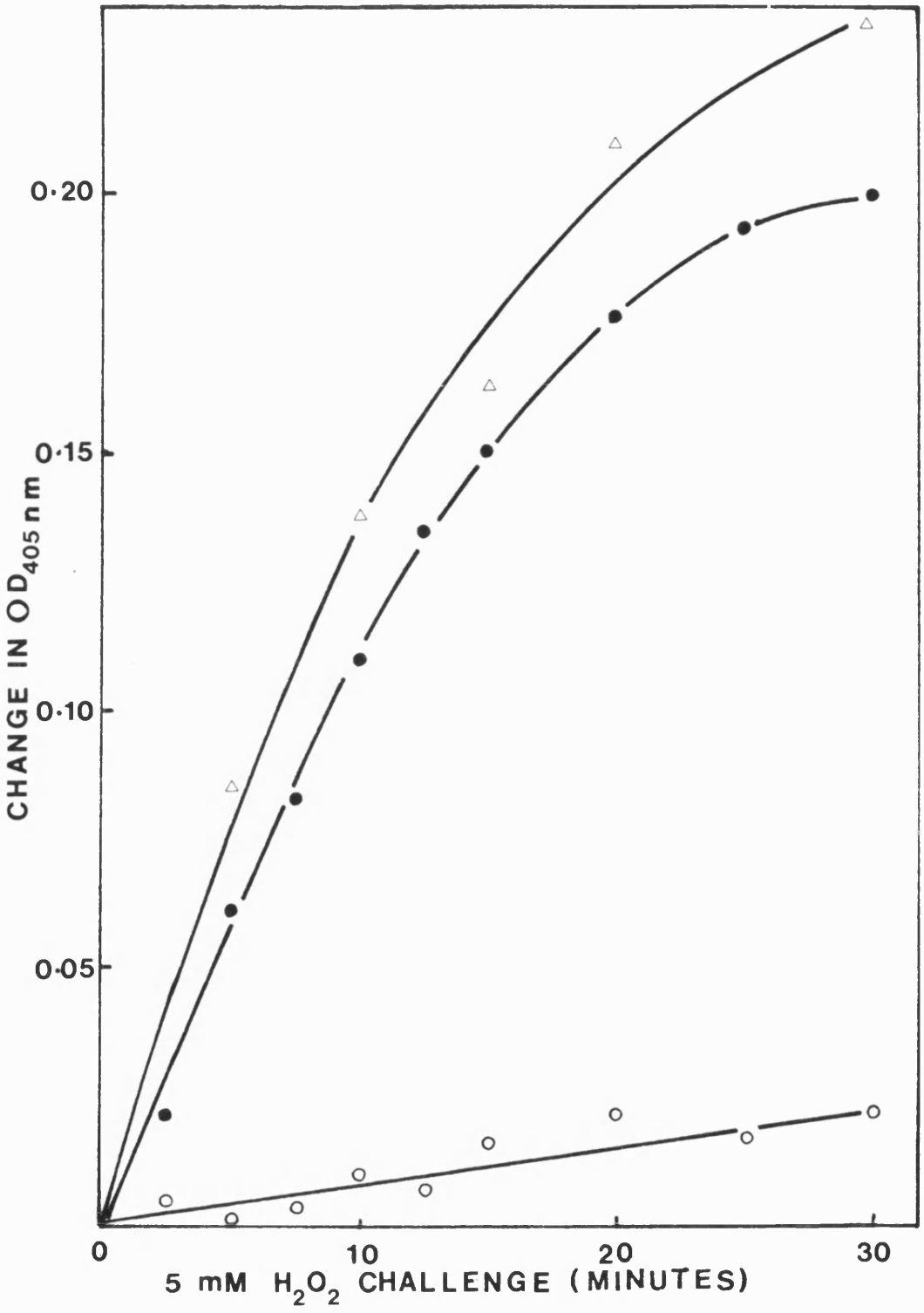


Figure 57: The H_2O_2 scavenging ability of stationary phase *E. coli* SR246 (●), *E. coli* UM1 (○) and *E. coli* SR385 (△) challenged with 5 mM H_2O_2.

Effect of Phase of Growth

The results presented so far in this section were all obtained using cells in the stationary phase of growth (i.e. 24 h cultures). The effect of the phase of growth on near-UV radiation sensitivity has been discussed in the general introduction and in Section II of the results. Since it was established that near-UV radiation sensitivity is dependent on the phase of growth and the response is strain specific, experiments were performed to establish whether SR246 is more or less sensitive to near-UV radiation in the stationary phase of growth as compared to the exponential phase of growth.

Stationary phase cells were obtained from 24 h culture. Exponential phase cells were obtained by inoculating 100 ml prewarmed YENB with 1 ml 24 h culture and growing the cells for 3½ h at 37°C until the mid-exponential phase of growth had been reached. Reference to a growth curve for SR246 (Appendix A, fig. 4) showed this to be when the optical density of the culture had reached approximately 0.1 (OD_{470}). Figure 58 shows the effect of phase of growth on the near-UV radiation sensitivity of SR246. Cells from the mid-exponential phase of growth are considerably more resistant to near-UV radiation than stationary phase cells, with a shoulder region covering the first 30 min of irradiation before a steady increase in sensitivity.

Discussion

The results presented in figs. 50-58 help to partially characterize the nature of the near-UV radiation sensitivity seen in *E. coli* SR246. SR246 shows near-UV radiation sensitivity after certain pre-irradiation growth conditions; cells must be in stationary phase and grown in a rich media such as YENB or nutrient broth to show sensitivity. From the

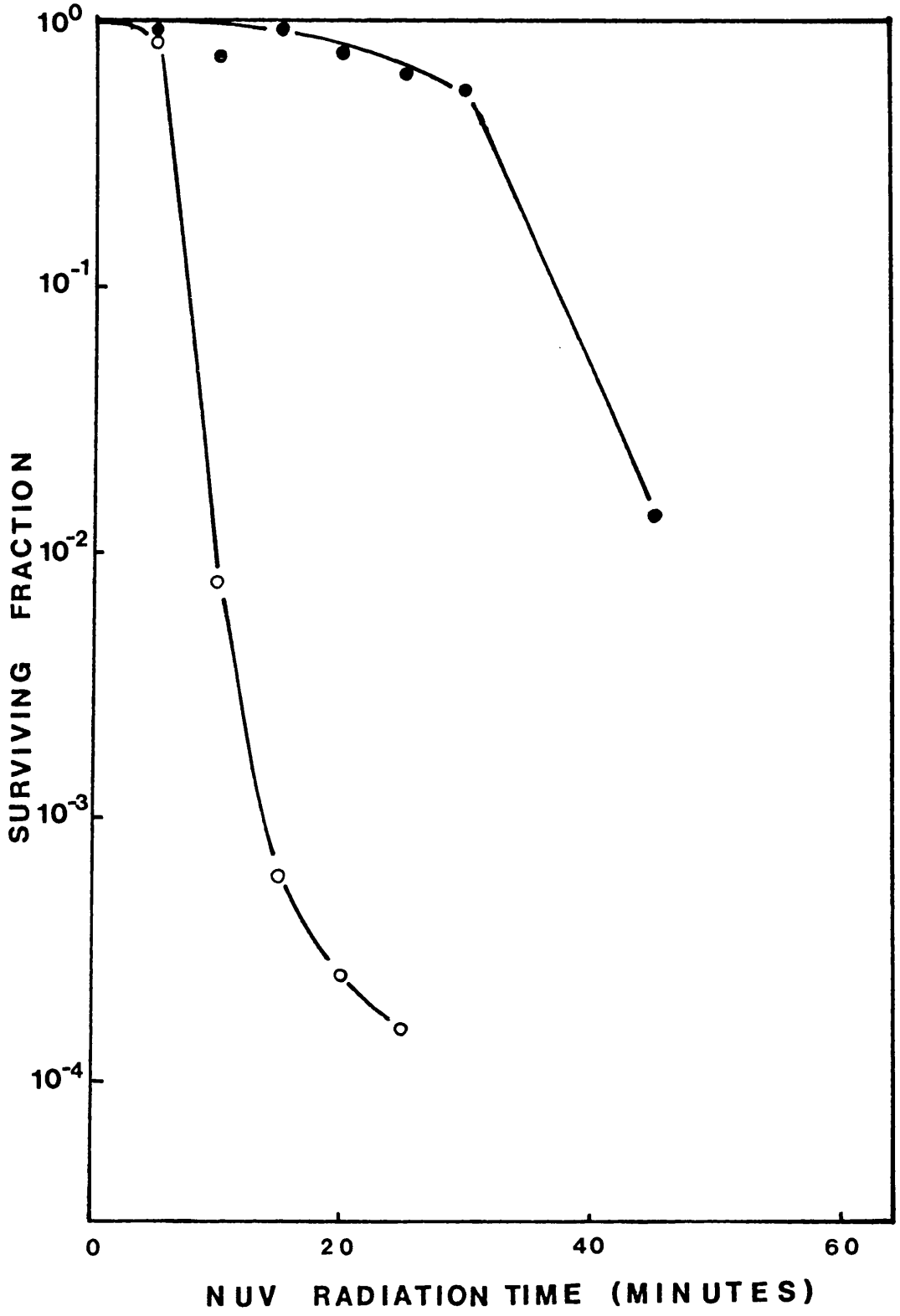


Figure 58: Survival curves for *E. coli* SR246 after near-UV irradiation. Stationary phase (○) and exponential phase (●) cells were grown in YENB and survival assessed on YENB plates.

experiments described here, it is not possible to determine why certain pre-irradiation growth conditions lead to near-UV radiation sensitivity. However, there are indications, that will be discussed further on, that membrane damage plays an important role and differences in both growth media and phase of growth would lead to variations in membrane composition. An alternative explanation is that when cells are grown for long periods in rich media (i.e. to stationary phase in broth), biosynthetic operons are repressed and the corresponding enzyme levels reduced (Ingraham *et al.*, 1983). This may mean that under these conditions, cells are unable to rapidly respond to and repair near-UV radiation induced damage; this would not be seen in exponentially growing cells or cells grown in a minimal media, as was shown experimentally (figs. 50 and 58). However, if this hypothesis is correct, it would not appear to be a general phenomenon as strains other than SR246 grown to stationary phase in rich media are not near-UV radiation sensitive (see SR385 control on fig. 51).

Post-irradiation growth conditions seem to be less important than pre-irradiation growth conditions for near-UV radiation sensitivity in SR246. Cells plated on YENB, TA or defined media showed similar responses to near-UV radiation after pre-irradiation growth in defined media (resistant response, fig. 50) or after pre-irradiation growth in YENB (sensitive response, fig. 51). The exception to this was seen when cells were grown in defined media and plated on nutrient agar (Oxoid) (fig. 52). In this case, cells were more sensitive than expected and it was suggested that this was due to the high salt content of Oxoid nutrient agar. Kelland *et al* (1983b) related near-UV radiation induced salt-sensitivity to membrane damage. It is possible that under the growth conditions used here (stationary phase, rich media), SR246 is susceptible to membrane damage. This membrane damage becomes more apparent if cells are plated on a media with high salt content.

The experiments that involved the use of rose bengal to generate singlet oxygen ($^1O_2$) would also suggest a role for membrane damage. When SR246 was compared to two *E. coli* strains that were resistant to near-UV radiation, it was found that SR246 was also more sensitive to 548 nm irradiation in the presence of rose bengal than the other two strains (fig. 54). In these experiments, the 548 nm illumination of rose bengal generates $^1O_2$ and since the rose bengal is unable to diffuse away from the beads to which it is bound, it is likely that the $^1O_2$ will preferentially attack the outer surface of the cell, that is the cell membrane. If the membrane is particularly sensitive to damage, due to particular growth conditions, the cells will be sensitive to rose bengal generated $^1O_2$, as was seen experimentally in fig. 54.

These ideas are supported by observations of Bezman *et al.*, 1978. Using similar experimental procedures, Bezman *et al.* (1978) showed that *E. coli* cells were inactivated by rose bengal immobilized on polypropylene beads and inactivation was due to photo-generated $^1O_2$. They supported these observations by showing that the inactivation rate increased significantly in D_2O, as compared to H_2O; D_2O is known to prolong the lifetime of $^1O_2$. Bezman *et al.* (1978) also showed that rose bengal was immobilized on the beads and not leaching out into solution, by preparing a suspension of rose bengal beads in buffer 2 h before an experiment, then filtering the suspension and using the filtrate only during irradiation, the survival curve obtained using the filtrate showed negligible inactivation of *E. coli* cells as compared to that obtained in the presence of rose bengal beads.

The results shown in fig. 55 show that SR246 is more sensitive to rose bengal generated $^1O_2$ after pre-irradiation growth in rich media rather than pre-irradiation growth in defined media. This is in agreement with the

near-UV radiation sensitivity results. However, the effect with $^1O_2$ is not so marked as that seen after near-UV irradiation. It may be that SR246 is intrinsically susceptible to membrane damage and that under certain conditions this damage is enhanced. The protection afforded by catalase in the plating medium after near-UV irradiation (fig. 56) would also indicate that there is a role for oxidative damage in SR246 near-UV radiation sensitivity. However, as discussed in Section I of the results, it is difficult to relate the effect of catalase in the plating medium to a specific damaging species and it may be that catalase is acting as a general antioxidant. It is unlikely that added catalase is replacing a catalase deficiency in SR246 since the H_2O_2 scavenging assay (fig. 57) demonstrates that SR246 shows considerable H_2O_2 scavenging ability and thereby, indirectly, catalase activity, when compared to the catalase deficient mutant, UMI.

PART II: Genetic mapping of near-UV radiation sensitivity of SR246

Bacterial Strains

The Hfr-Tn10 strains used in the mapping experiments and listed in fig. 59, were obtained from Dr B. Bachmann (*E. coli* Genetic Stock Centre, U.S.A.). The sixteen Hfr-Tn10 mapping strains were constructed by Wanner (1986). A summary of the Hfr points of origin and direction of transfer is included in fig. 59 and a complete strain description is to be found in Appendix A, Table 1.

Materials

1) **Media**

Selective plates used in mapping experiments were either based on defined media plates (as described in the general methodology) for the strain in use, with the appropriate antibiotics added, or streptomycin, tetracycline nutrient agar plates (ST plates). These were prepared by dissolving nutrient broth (10.4 g, Oxoid) and agar technical number 3 (9.6 g, Oxoid) in 800 ml distilled water and sterilized by autoclaving at 121°C for 20 min. After sterilizing, the media was allowed to cool to approximately 60°C and transferred to a laminar air flow hood before adding streptomycin (final concentration 100 µg/ml) and tetracycline (final concentration 15 µg/ml). The plates were poured as described previously.

2) **Antibiotics**

Streptomycin and tetracycline were used in this section as selective agents in the mapping experiments.

Streptomycin was prepared by dissolving streptomycin sulphate (10

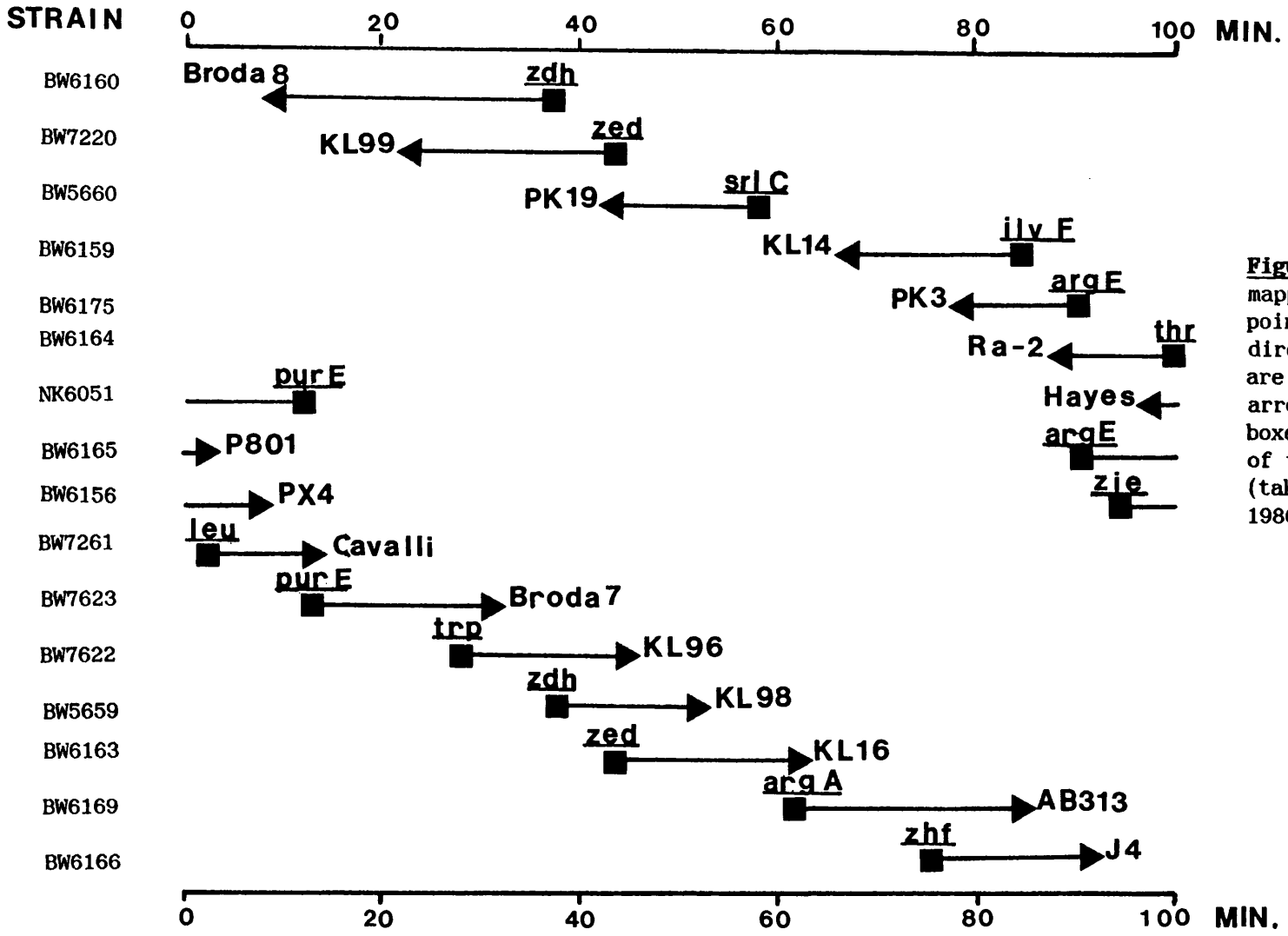


Figure 59: Hfr-Tn10 mapping strains. The Hfr points of origin and direction of transfer are shown by the arrowheads. The filled boxes show the position of the Tn10 insertions (taken from Wanner, 1986).

mg/ml, Sigma) in distilled water. This was filter sterilized and stored at 4°C. This stock solution was diluted as required so as to give a final concentration of 100 µg/ml.

Tetracycline was prepared by dissolving tetracycline hydrochloride (50 mg/ml, Sigma) in 95% ethanol which was then made up to volume with distilled water in a 50/50 ratio. This was filter sterilized and stored at 0°C in the dark. This stock solution was diluted as required so as to give a final concentration of 15 µg/ml.

Methods

Method for Spot Conjugation

A simple bacterial mating method, based on that of Miller (1972) was used to obtain preliminary results from crossing SR246 with the sixteen Hfr-Tn10 strains. Donors and recipient were grown at 37°C overnight in 5 ml YENB. A 0.05 ml aliquot of the overnight culture was sub-cultured to 5 ml prewarmed YENB and grown at 37°C for 75 min. Selective ST plates were overdried for 2 h and marked into three areas. A 0.02 ml aliquot of the recipient was spotted onto two of the three areas. After these spots had been absorbed by the agar, a 0.02 ml aliquot of the donor was spotted to the third area and also on top of one of the recipient spots. Once these spots had been absorbed by the agar, the plates were incubated for 48 h at 37°C. Growth was observed in the mixed spots where mating had occurred. The other two unmixed spots were used as controls for revertants.

Interrupted Mating Method

Interrupted mating experiments were based on the method described by Miller (1972). The donors and recipient were grown overnight at 37°C in

5 ml YENB. Cells were subcultured in 50 ml prewarmed YENB and grown in $2x10^8$ CFU/ml. The donors were grown at 37°C in a non-shaking water bath to aid the formation of F-pili. Donor and recipient were gently mixed in the ratio of 1:10 in a 250 ml conical flask, and left at 37°C. At the appropriate times 0.2 ml samples were taken, added to 3.8 ml cold M9 salts solution and vortexed on a bench-top whirlimixer for 20 seconds to separate mating pairs. This suspension was then diluted to the appropriate level and 0.2 ml samples plated onto selective media. The plates were incubated at 37°C for 48 h. Two controls were also run; (1) Control for revertants: 0.2 ml samples of donor and recipient were taken before mixing, diluted, plated out separately onto selective media and incubated as above; (2) Zero control: a 0.2 ml sample was taken immediately after mixing, diluted, vortexed and plated as above. This was to ascertain that mating pairs had been adequately separated. The experimental conditions used, i.e. cell density, mixing ratio of donor to recipient and aeration (which was achieved by having a large surface area, 10 ml mating mix in a 250 ml flask, rather than by agitation which may prevent the cells from forming mating pairs) were chosen to give maximum mating efficiency.

Method for Near-UV Screening

The following method was developed to test the near-UV sensitivity of the SR246 x Hfr-Tn10 recombinants. A simple method was needed to enable the rapid screening of recombinants so that their near-UV radiation sensitivity could be compared to both SR246 and the relevant parent strains.

Primary cultures were obtained by inoculating 5 ml YENB, in test tubes, and growing the cells overnight at 37°C. Stationary phase cultures were obtained by inoculation of 5 ml prewarmed YENB with 0.05 ml of the overnight culture and growing at 37°C for 24 h. Cells were harvested by

spinning down 1 ml of the 24 h stationary phase culture in a bench-top microcentrifuge (MSE, microcentaur) at high speed (1300 rpm) for 1 min. Cells were washed, resuspended in 10 ml M9 salts solution and diluted to give 10^7 CFU/ml. Five millilitre samples of these suspensions were irradiated with broad-band near-UV radiation. Samples were taken immediately before irradiation and after 30 min irradiation, serially diluted in M9 salts solution and plated on YENB agar. Plates were incubated at 37°C for 48 h. Usually, six samples were irradiated simultaneously and each group included both SR246 and the appropriate parent strain. Cell suspensions were kept at room temperature whilst awaiting irradiation. Apart from acting as comparisons against which the near-UV radiation sensitivities of the recombinant strains could be checked, the inclusion of SR246 and the parent strains in each run acted as a control to ensure that the near-UV radiation sensitivity of the strains that had been waiting at room temperature had not altered.

Nomenclature

In this section of results, the following nomenclature has been used; SR246 and the Hfr-Tn10 parent strains retain their own strain names. Conjugants made by crossing SR246 with Hfr-Tn10 strains are referred to as X1, X2, X3, etc., where the number distinguishes the parent donor. The key for the numbers used is:

1	BW6160	9	BW6156
2	BW7220	10	BW7261
3	BW5660	11	BW7623
4	BW6159	12	BW7622
5	BW6175	13	BW5659

6	BW6164	14	BW6163
7	NK6051	15	BW6169
8	BW6165	16	BW6166

With the interrupted mating experiments, samples taken at different times are identified by adding the sampling time to the conjugant number, for example when SR246 and BW6160 are crossed and the mating interrupted after 35 min, the resultant conjugants would be referred to as X1/35.

For the remainder of the experiments to be described in this results section, cells used were grown under conditions that lead to maximum near-UV radiation sensitivity; that is, 24 h stationary phase cells grown in YENB. Unless otherwise stated, cells have been plated onto YENB agar plates.

Results

Hfr-Tn10 Strains

The sixteen Hfr-Tn10 strains were tested on defined media for their growth requirements, to verify their phenotypes. The point of origin and direction of transfer were also determined. *Escherichia coli* K12 AB1157 was used as the recipient since SR246 has few nutritional markers that could be followed. Interrupted mating experiments were carried out between the donors and K12 A1157. Recombinants were plated onto different selective, defined media plates. For example, BW5659 was conjugated with K12 AB1157. Samples were taken every two minutes over a 30 min period, diluted and plated onto (a) defined media with growth requirements for K12 AB1157, with the exception of histidine to prevent the growth of A1157, and with the addition of streptomycin to prevent the growth of BW5659; and (b) defined media with the growth requirements for AB1157, with the exception

of histidine but with the addition of tetracycline to prevent the growth of AB1157 and early conjugants respectively and with the addition of streptomycin, to prevent BW5659 growth. Only conjugants that had received *his*$^+$ at 44' are able to grow on media (a), whilst only those that have received tetracycline resistance at 37' will grow on media (b). The number of recombinants per ml was plotted against the length of conjugation. The results of this experiment and a similar experiment using BW6163, are shown in figs. 60 and 61. From these results, an estimate of the relative time of entry of markers and Tn10 insertions can be made and the order in which markers appear indicates the direction of transfer. The Hfr-Tn10 points of origin, direction of transfer and position of Tn10 insertion of each Hfr-Tn10 strain used in this section were checked by AB1157 x Hfr-Tn10 crosses as described above. The results shown in figs. 60 and 61 are representatives of the type of data obtained.

Reproducibility of Near-UV Radiation Screening Method

Figure 62 shows the result of screeing 9 replicate samples of SR246 for near-UV radiation sensitivity. Samples were taken after 15 min and 30 min near-UV irradiation. The nine samples were tested as two groups of five and four samples respectively. The results presented in fig. 62 show that the near-UV radiation screening method used gives a reasonably reproducible result.

Preliminary Data on Mapping the Near-UV Radiation Sensitivity of SR246

Near-UV Radiation Sensitivity of Hfr-Tn10 Strains

The near-UV radiation sensitivity of the 16 Hfr-Tn10 strains was determined using the near-UV screening method. Twenty-four hour, stationary phase cells grown in YENB were used. After harvesting and

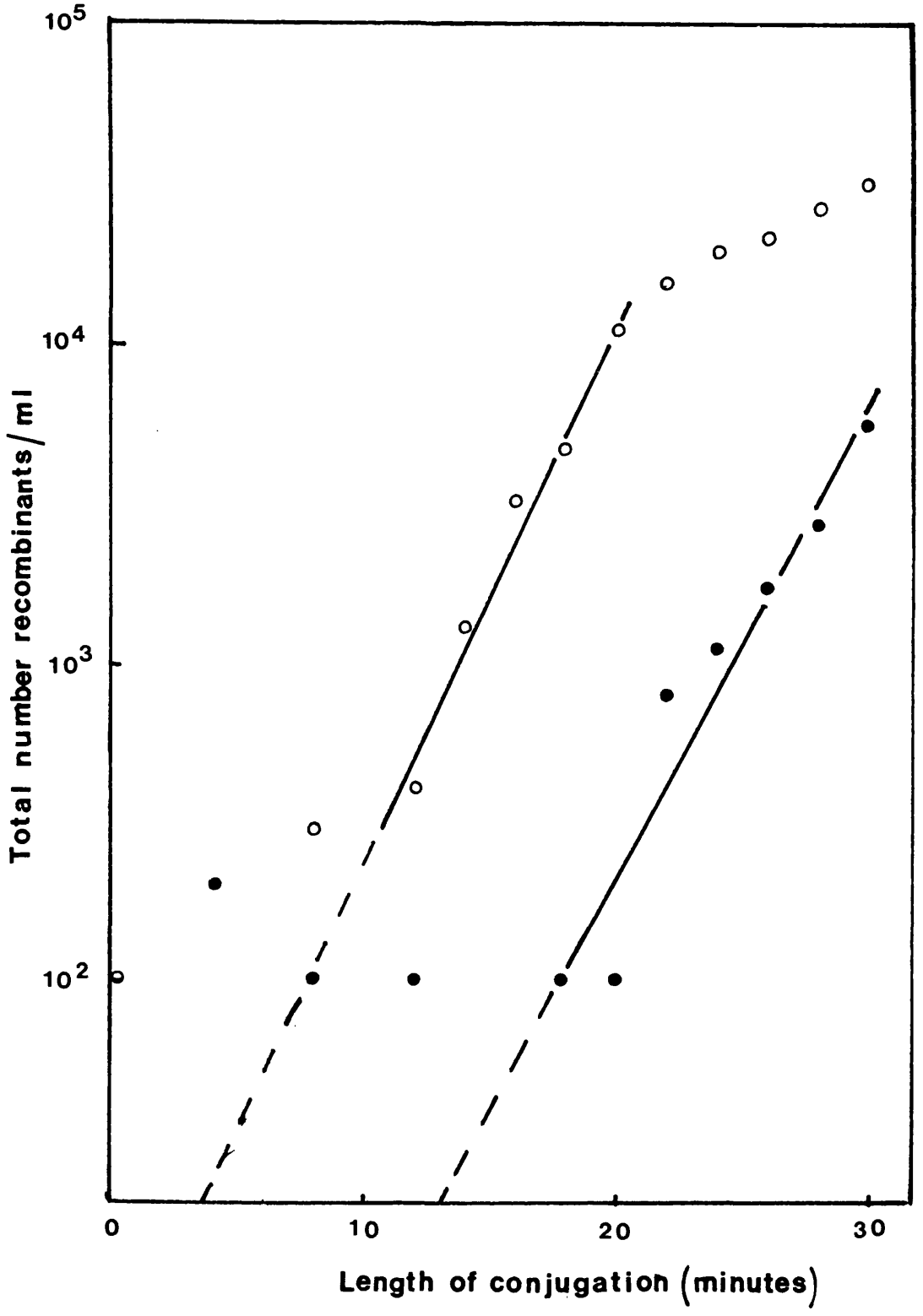


Figure 60: The number of *E. coli* K12 AB1157 x BW5659 recombinants after interrupting conjugation at the times shown on the abscissa. Viability was assessed on his^-, str^+ defined media (o) and his^-, str^+, tet^+ defined media (●). The lines have been drawn by eye through the steepest portions of the curves.

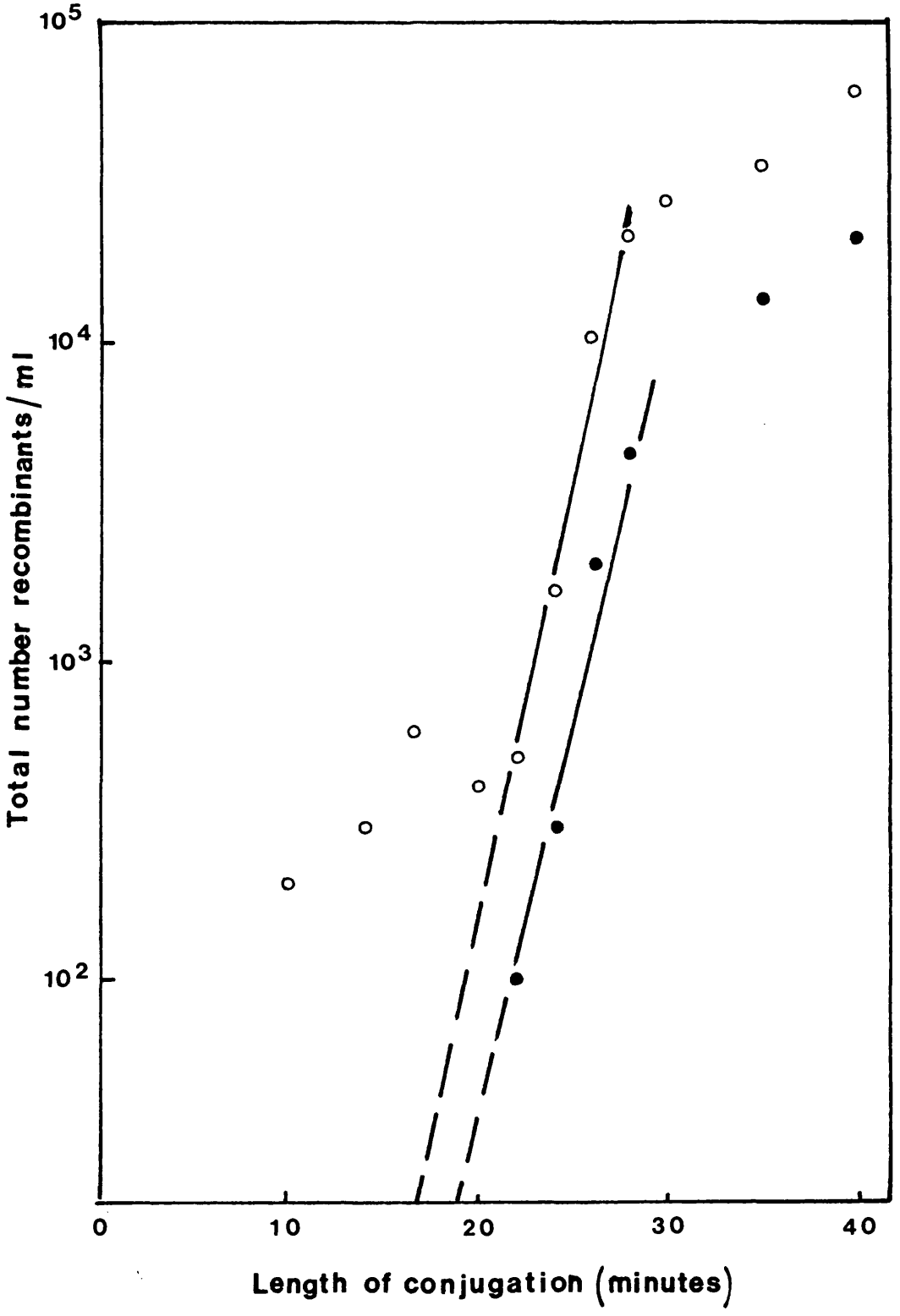


Figure 61: The number of *E. coli* K12 AB1157 x BW6163 recombinants after interrupting conjugation at the times shown on the abscissa. Viability was assessed on his^-, str^+ defined media (o) and his^-, str^+, tet^+ defined media (●). The lines have been drawn by eye through the steepest portions of the curves.

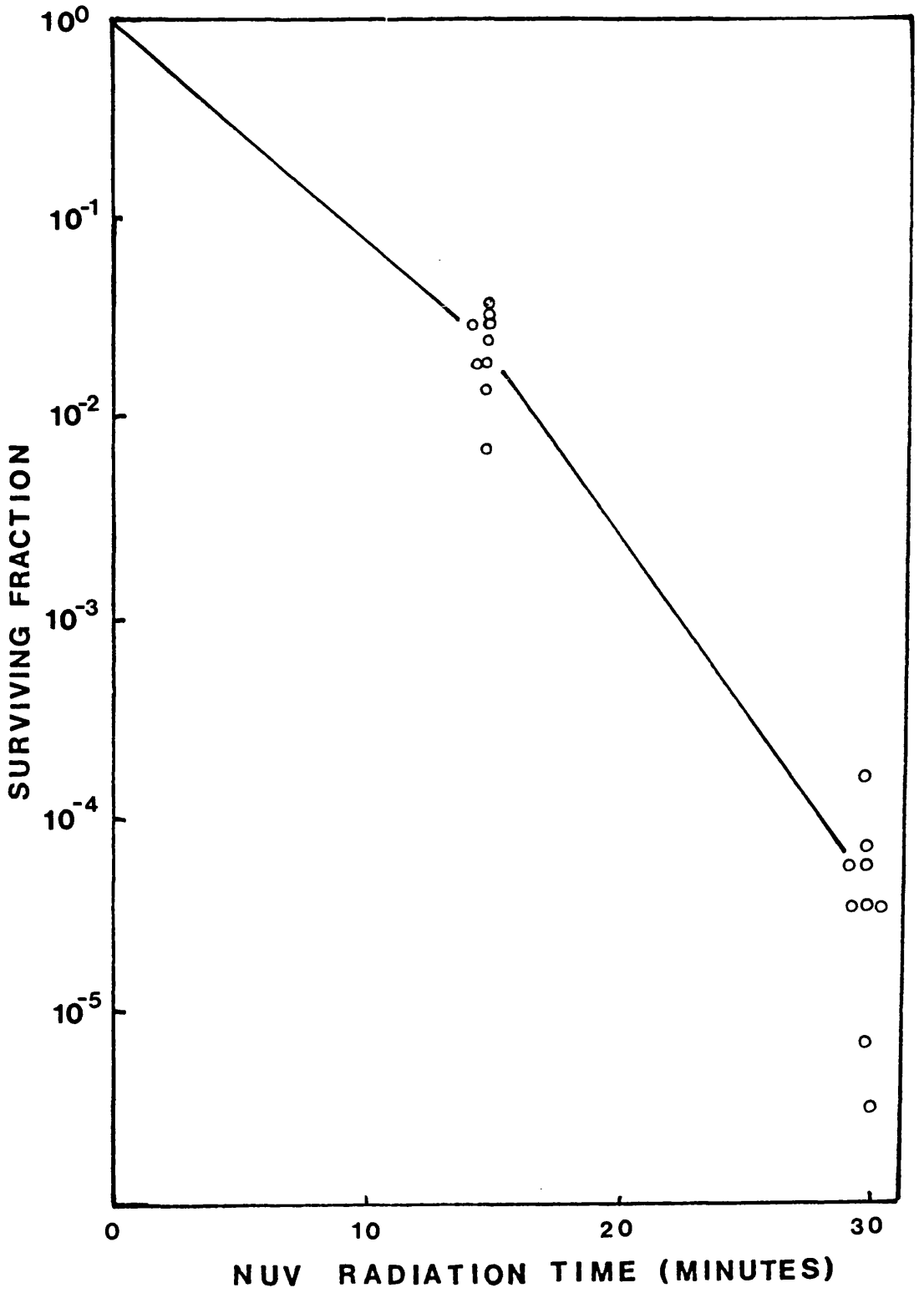


Figure 62: Replicate surviving fractions for *E. coli* SR246 after near-UV irradiation with viability assessed on YENB plates. Nine replicates were irradiated at each time and the mean points joined to give the survival curve.

preparing the cell suspensions, the Hfr-Tn10 strains were randomly divided into three groups (containing 5, 5 and 6 strains) and irradiated for 30 min. Each groups also contained a sample of SR246 for comparison and as an internal control. The results are shown in fig. 63. Each point represents the mean of two identical experiments. Eleven of the 16 strains showed very little decrease in viability after 30 min near-UV radiation (BW6164, BW6163, BW7261, BW7622, BW6169, BW6159, BW7220, BW5660, BW6156, BW6175, BW5659). A further four strains (NK6051, BW7623, BW6160, BW6165) showed 1-2 log kill after this period of irradiation. Only one strain (BW6166) showed marked near-UV radiation sensitivity, but even this strain was less sensitive than SR246.

Spot conjugations were carried out between the Hfr-Tn10 strains and SR246. Conjugants from the successful matings were taken, grown to 24 h stationary phase in YENB and screened for near-UV radiation sensitivity. The results are shown in fig. 64. Of the conjugants tested, three (X9, X15, X14) showed considerably increased resistance to near-UV radiation when compared to SR246. The remaining six conjugants showed little or no difference from SR246.

This method gives an approximate indication of the regions of the chromosome that might be involved in SR246 near-UV radiation sensitivity. However, since the strains are mixed and left together during incubation, there is no indication as to the length of conjugation, although the probability of long regions of the chromosome being transferred intact is low. Therefore conjugation was followed more closely by interrupted mating experiments.

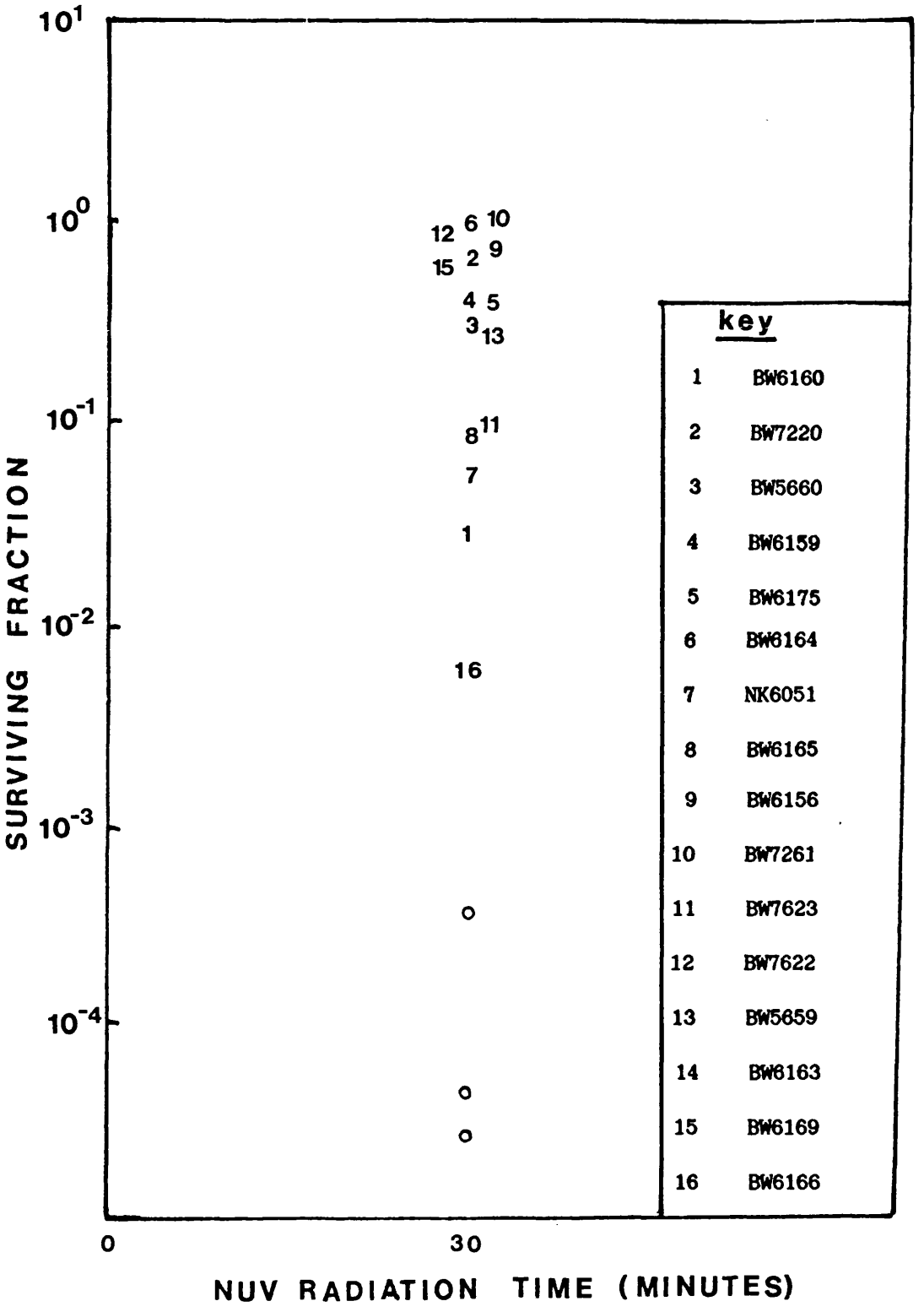


Figure 63: Surviving fractions of *E. coli* SR246 (o) and 16 Hfr-Tn10 parent strains (1,2,etc) after 30 min near-UV irradiation. Points have been displaced horizontally where necessary to prevent obscuring numbers.

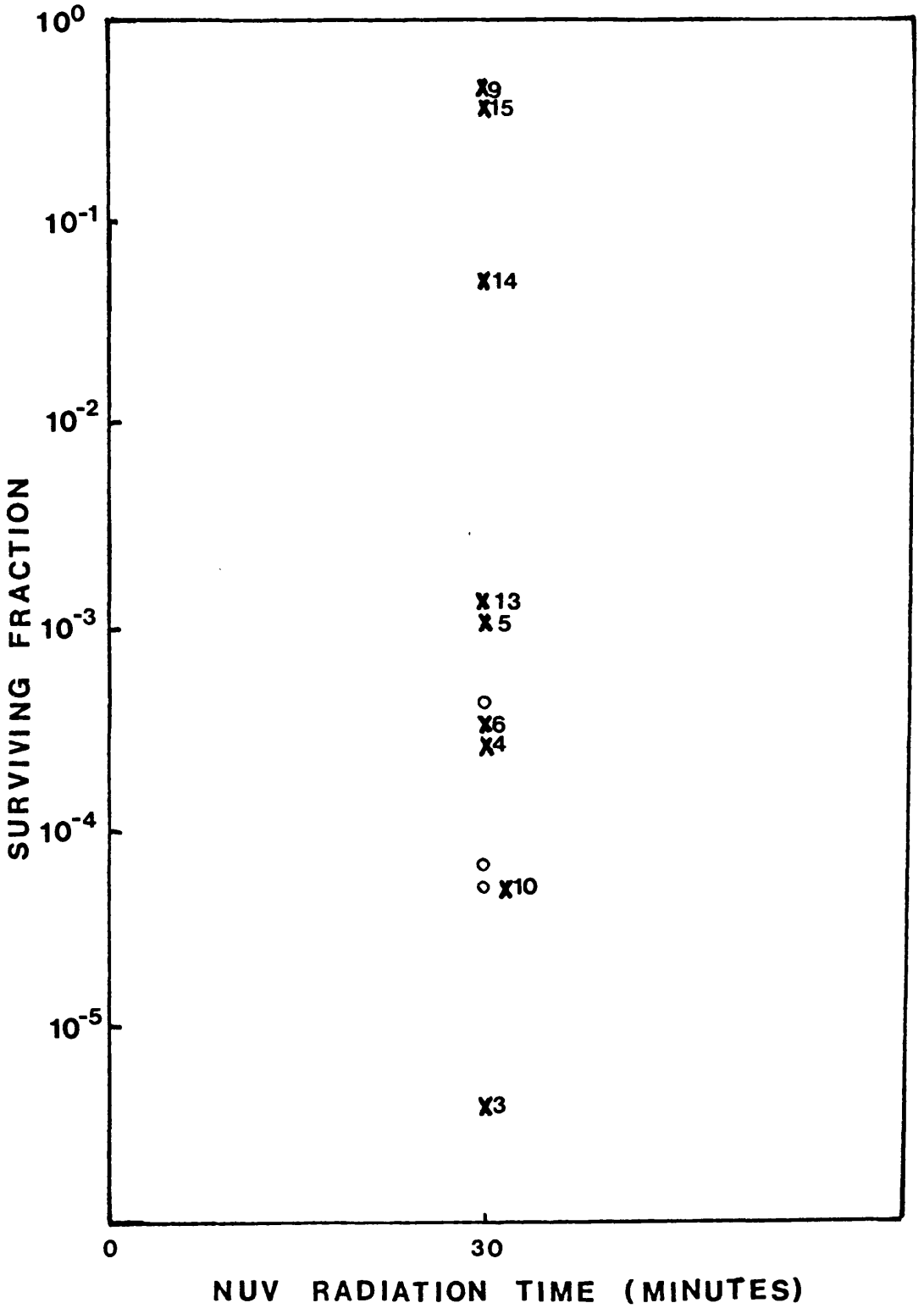
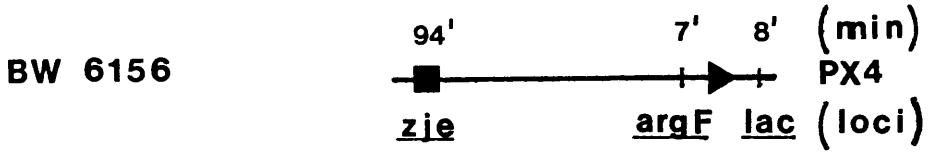


Figure 64: Surviving fractions of *E. coli* SR246 (o) and 9 246 x Hfr-Tn10 conjugants (X9,X15, etc) after 30 min near-UV irradiation. Points have been displaced horizontally where necessary to prevent obscuring numbers.

Near-UV Radiation Sensitivity of SR246 x BW6156 (X9) Conjugants

The point of origin, direction of transfer and position of Tn10 insertion of BW6156 are represented diagramatically below:



In this and similar diagrams, the arrowhead represents the Hfr point of origin and direction of transfer and the filled box shows the position of Tn10 insertions.

Nine replicate colonies obtained from the spot mating experiment of SR246 x BW6156 which had shown near-UV radiation resistance (fig. 64) were patched out on ST agar selective plates and grown overnight at 37°C. Individual colonies were then grown to stationary phase in YENB and screened for near-UV radiation sensitivity. The results are shown in fig. 65, with SR246 and the donor parent strain BW6156 as comparisons. All nine conjugant replicates were resistant to near-UV radiation showing approximately the same response as the parent strain BW6156.

SR246 and BW6156 were conjugated using the interrupted mating method. SR246 has few nutritional markers so transfer of tetracycline resistance was used to follow conjugation. Transfer of tetracycline resistance occurs when the region of donor chromosome from the Hfr point of origin to the Tn10 insertion has been successfully integrated into the recipient chromosome. Figure 66 shows the result of an interrupted mating experiment between SR246 and BW6156. Tetracycline resistant conjugants begin to appear on the selective media after approximately 15 min of conjugation.

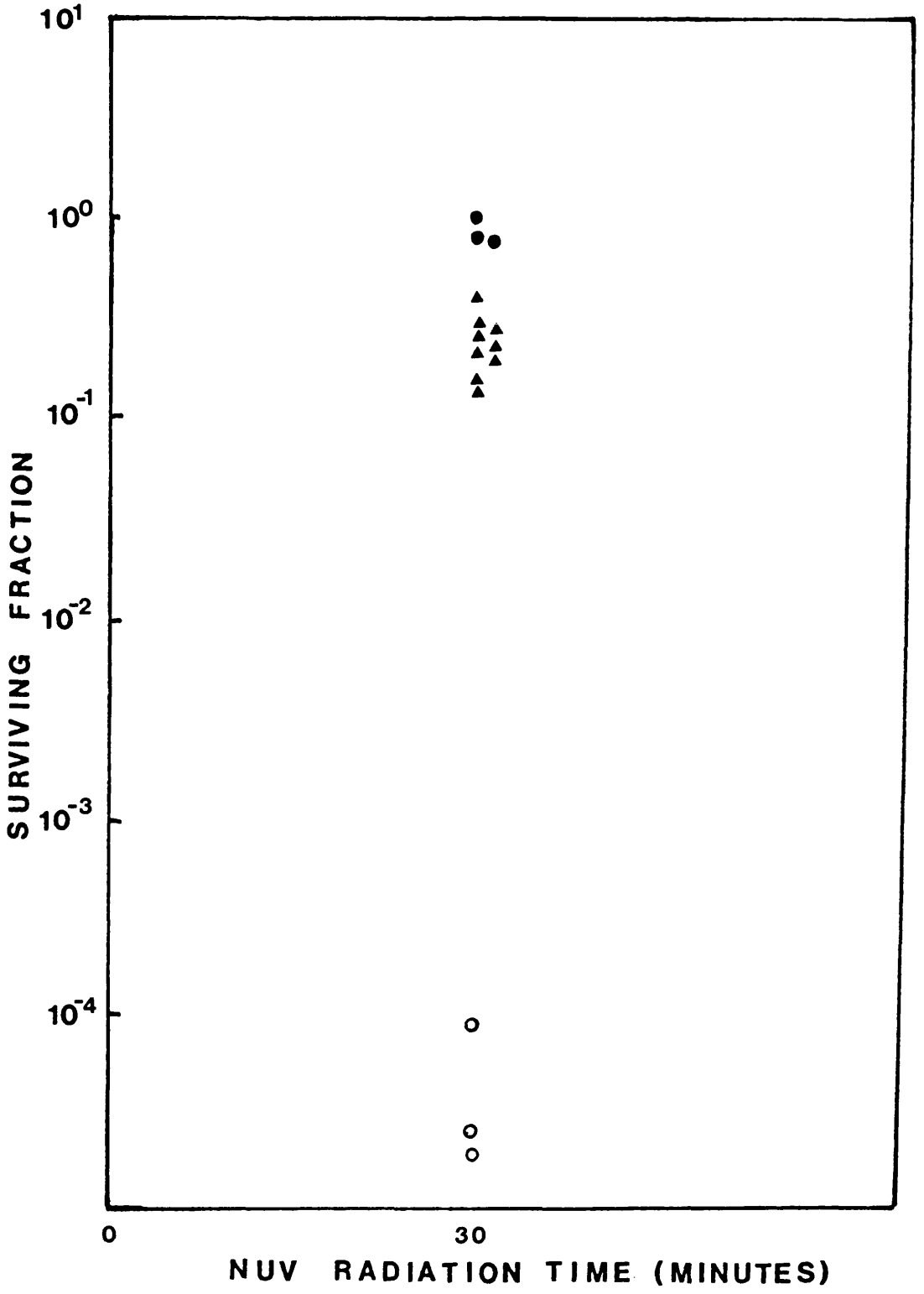


Figure 65: Surviving fractions of *E. coli* SR246 (o), the Hfr-Tn10 strain BW6156 (●) and the SR246 x BW6156 conjugant X9 (▲) after 30 min near-UV irradiation. Points have been displaced horizontally where necessary to prevent obscuring symbols.

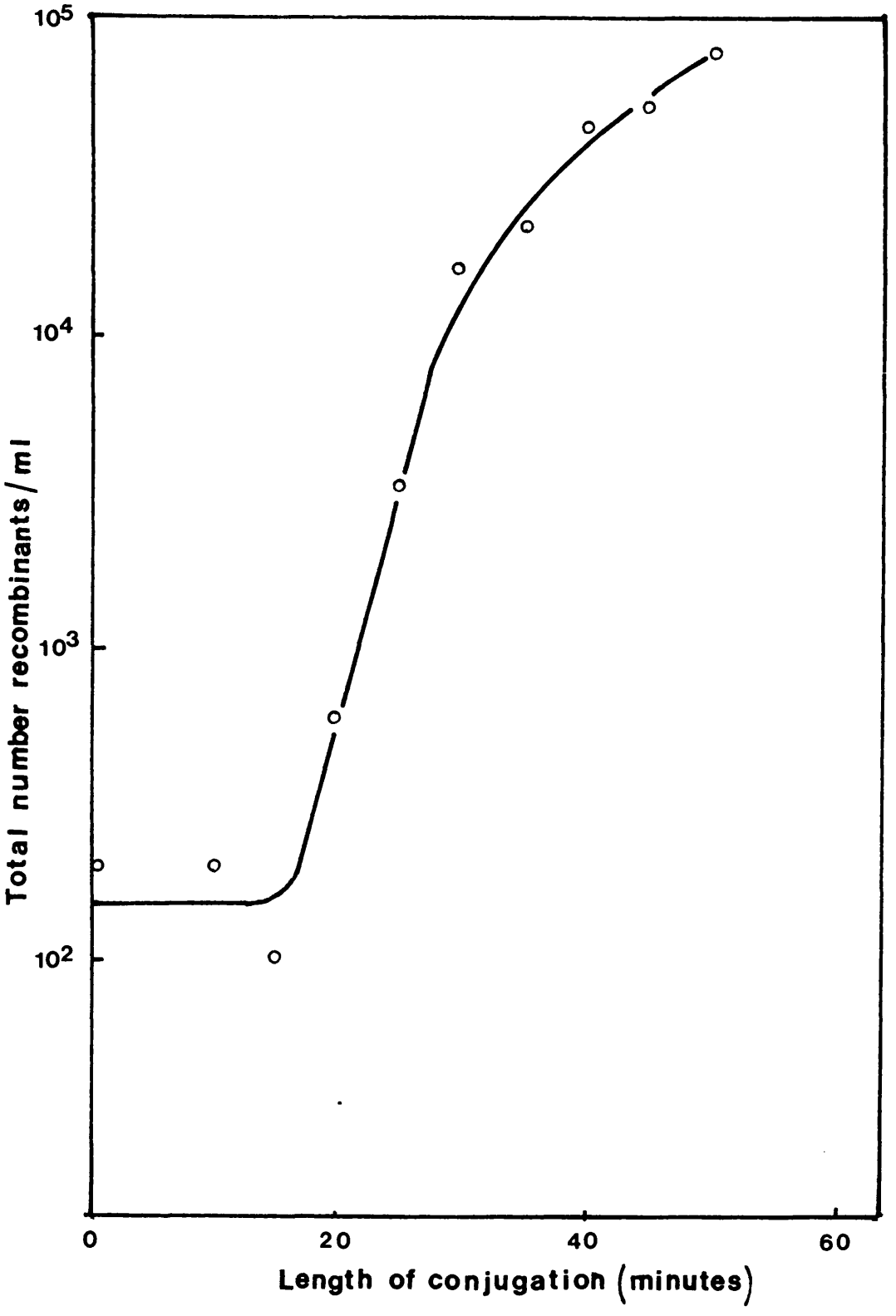


Figure 66: The number of X9 recombinants after interrupting conjugation between SR246 and BW6156 at the time shown on the abscissa. Viability was assessed on streptomycin/tetracycline nutrient agar.

This is to be expected as a period of time is required for the formation of stable mating pairs and the onset of DNA transfer, and there is a 13' period between the Hfr point of origin and the Tn10 insertion. The number of recombinants begins to plateau after approximately 35 min due to the increasing probability of spontaneous chromosome breakage with increasing length of conjugation. Single colonies were patched onto ST agar and grown overnight at 37°C, then grown to 24 h stationary phase in YENB and screened for near-UV radiation sensitivity. Since the spot mating experiment had given no indication of the duration of mating required to transfer near-UV radiation resistance to the recombinants, colonies were taken from plates which covered a 25 min time range of sampling. Conjugants were identified by labelling them with both the conjugant number and the time of sampling. Thus the conjugant X9/35 is from a SR246 x BW6156 cross, where mating was interrupted 35 min after mixing.

The results of screening SR246 x BW6156 (X9) conjugants for near-UV radiation sensitivity are shown in fig. 67. Conjugants taken from 25, 30, 35, 40 and 50 min plates showed little or no decrease in near-UV radiation sensitivity. These results are somewhat unexpected considering the result of the spot mating experiment (fig. 65). Conjugants from the 50 min plates represent cells in which, theoretically, approximately 63' of the donor chromosome could have been transferred. It is however likely that double or multiple crossovers would have occurred during this period of time. If the gene conferring SR246 near-UV radiation sensitivity is near to the end of the region on the recipient chromosome, the probability of it being replaced by the complimentary near-UV radiation resistant gene from this donor is low.

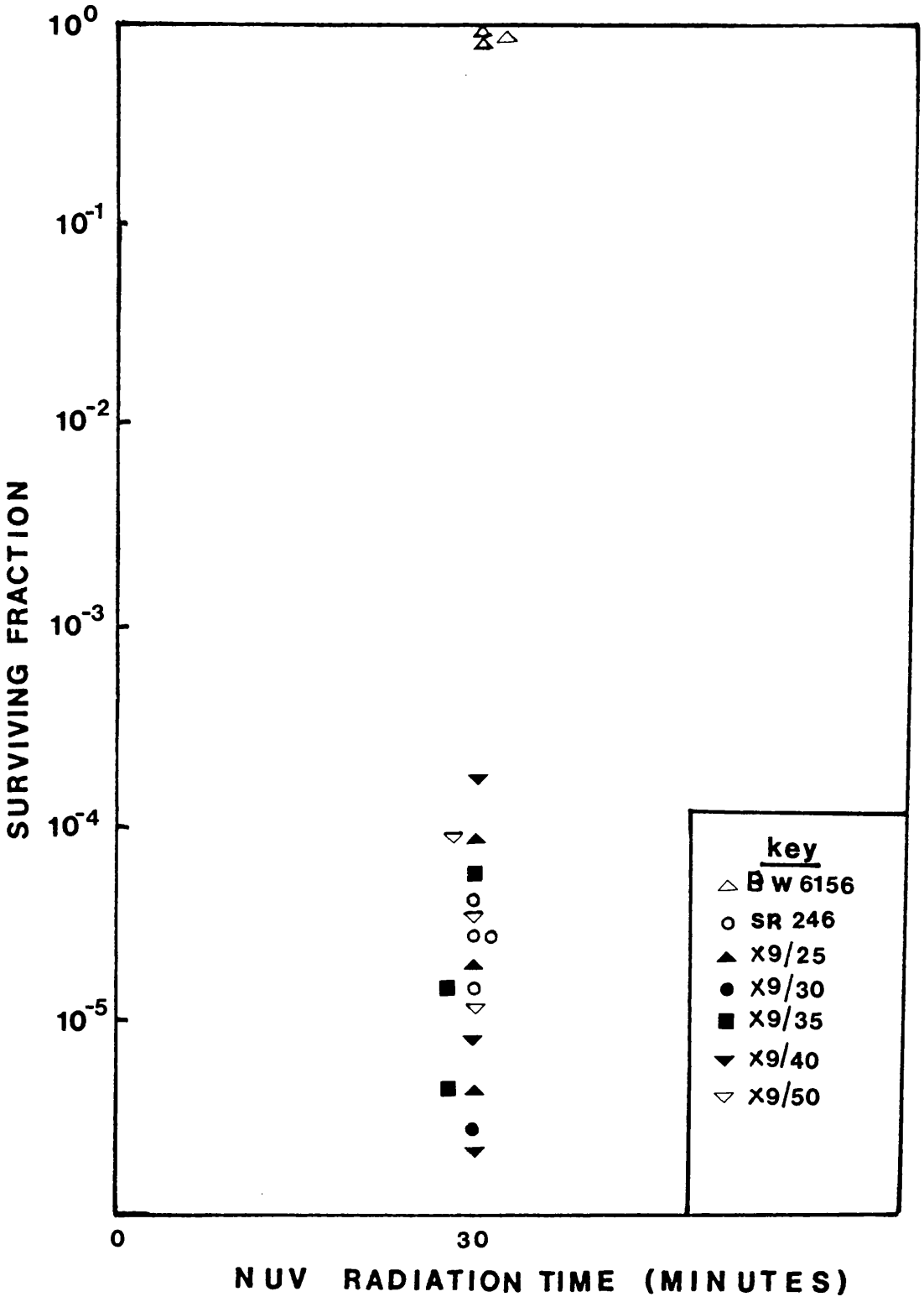


Figure 67: Surviving fractions for *E. coli* SR246 (o), BW6156 (△) and 13 X9 conjugants (as in key) after 30 min near-UV irradiation. Points have been displaced horizontally where necessary to prevent obscuring symbols.

Near-UV Radiation Sensitivity of Further SR246 x Hfr-Tn10 Conjugants

This same procedure of interrupted mating experiments followed by near-UV radiation sensitivity screening of conjugants was then used with other Hfr-Tn10 strains covering subsequent regions of the chromosome. Figure 68 shows details of the Hfr points of origin, direction of transfer and position of Tn10 insertion for the six strains used. In each case interrupted mating was carried out as described for BW6156. Conjugants obtained after varying lengths of conjugation were screened for near-UV radiation sensitivity and the results of all the screenings are shown in figs. 69 and 70.

Although the Hfr-Tn10 strain covering the next region of the chromosome after BW6156 (anticlockwise) is BW6166, this was not used as the near-UV sensitivity of this strain (fig. 63) is not much greater than SR246, which would make the near-UV radiation screening of any conjugants difficult to interpret. Instead, BW6169 was used as the next donor strain. The results of screening conjugants of the SR246 x BW6169 cross (X15) are shown in fig. 69A. Although the majority of the conjugants show similar near-UV radiation sensitivities to SR246, there is a tendency for later conjugants to be more near-UV radiation resistant. One conjugant taken 50 min after mixing (X15/50) had a response approaching that of the parent strain BW6169.

However, overall the results again do not agree with the result of the spot mating (fig. 63) which showed X15 to be near-UV resistant, although the result with one X15/50 conjugant would indicate that the near-UV radiation sensitivity of SR246 is located near the end of the region of chromosome transferred by BW6169.

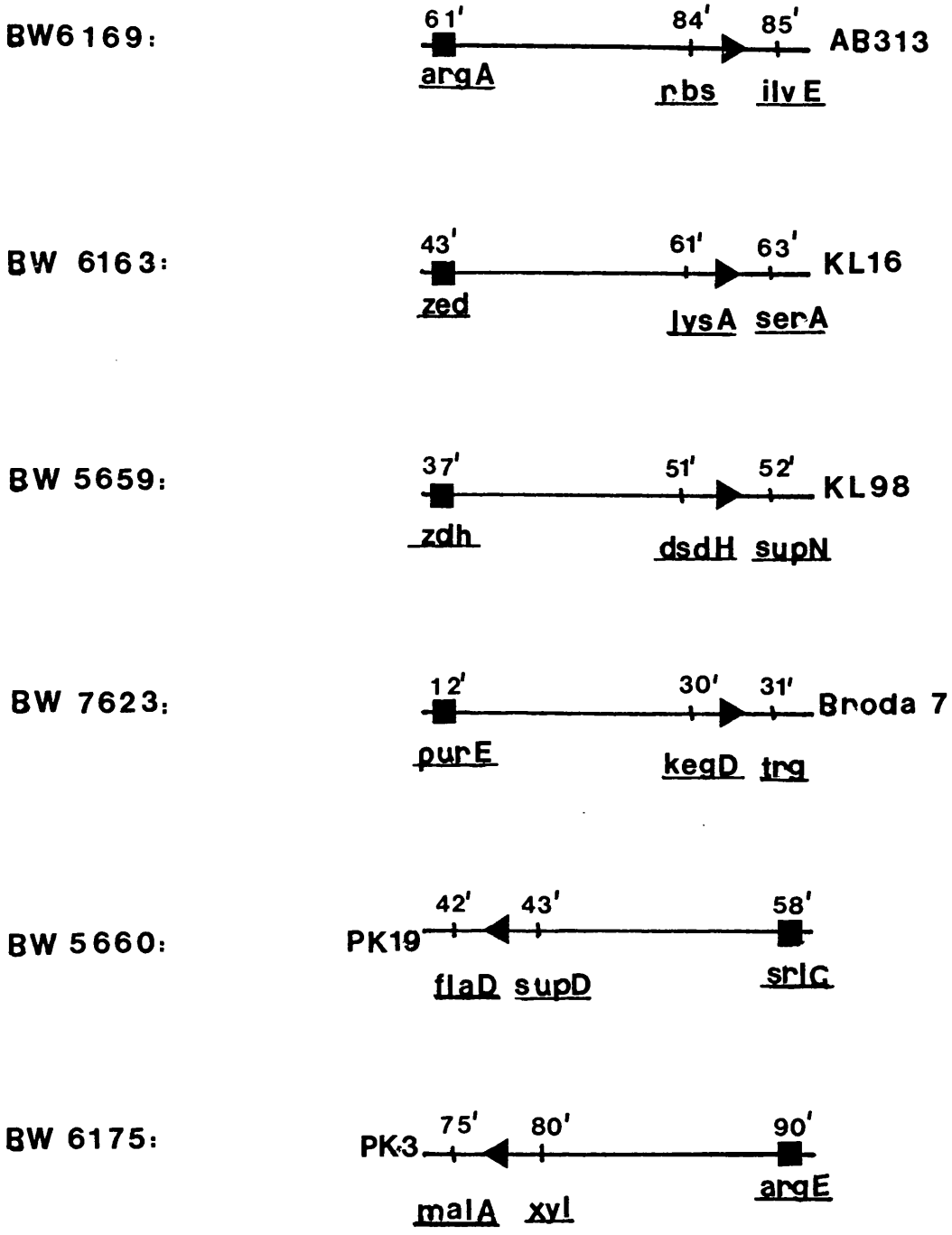


Figure 68: Hfr points of origin and direction of transfer (arrowheads) and positions of Tn10 insertions (box) for six Hfr-Tn10 strains.

For the same reasons given above for BW6156, it is possible that the near-UV radiation resistance is not transferred until late in the conjugation as is indicated by the result of fig. 69A. Therefore interrupted mating and near-UV radiation screening of conjugants was carried out with BW6163, which covers the next anticlockwise region of the chromosome. The results of screening the SR246 x BW6163 (X14) conjugants is shown in fig. 69B. Seven of the twelve conjugants tested showed a considerable increase in near-UV radiation resistance over SR246. Of these seven strains, four were from colonies taken after 50 min conjugation and the other three were from 30 min conjugation. Figure 69C shows the result of screening conjugants from SR246 x BW5659 cross (X13). Conjugants from both early (26 min) and late (50 min) in conjugation showed increased near-UV radiation resistance although none of the X13 conjugants showed as great a resistance as that seen in fig. 69B with X14 conjugants. One further strain covering a region in the anticlockwise direction was tested. This was BW7623 and the results of screening of the SR246 x BW7623 (X11) conjugants are shown in fig. 70A. This shows that these X11 conjugants have similar near-UV radiation responses as SR246. The results shown in fig. 69 and fig. 70A indicate that the region containing the near-UV radiation sensitivity of SR246 is covered by the Hfr-Tn10 strain BW6163. However, it is surprising that the result is not more clear-cut. To try and clarify the result, two complimentary Hfr-Tn10 strains covering a similar region but in a clockwise sense were also tested. These were BW5660 and BW6175 and the results obtained are shown in figs. 70B and C. No near-UV radiation resistance was observed in any of the conjugants tested.

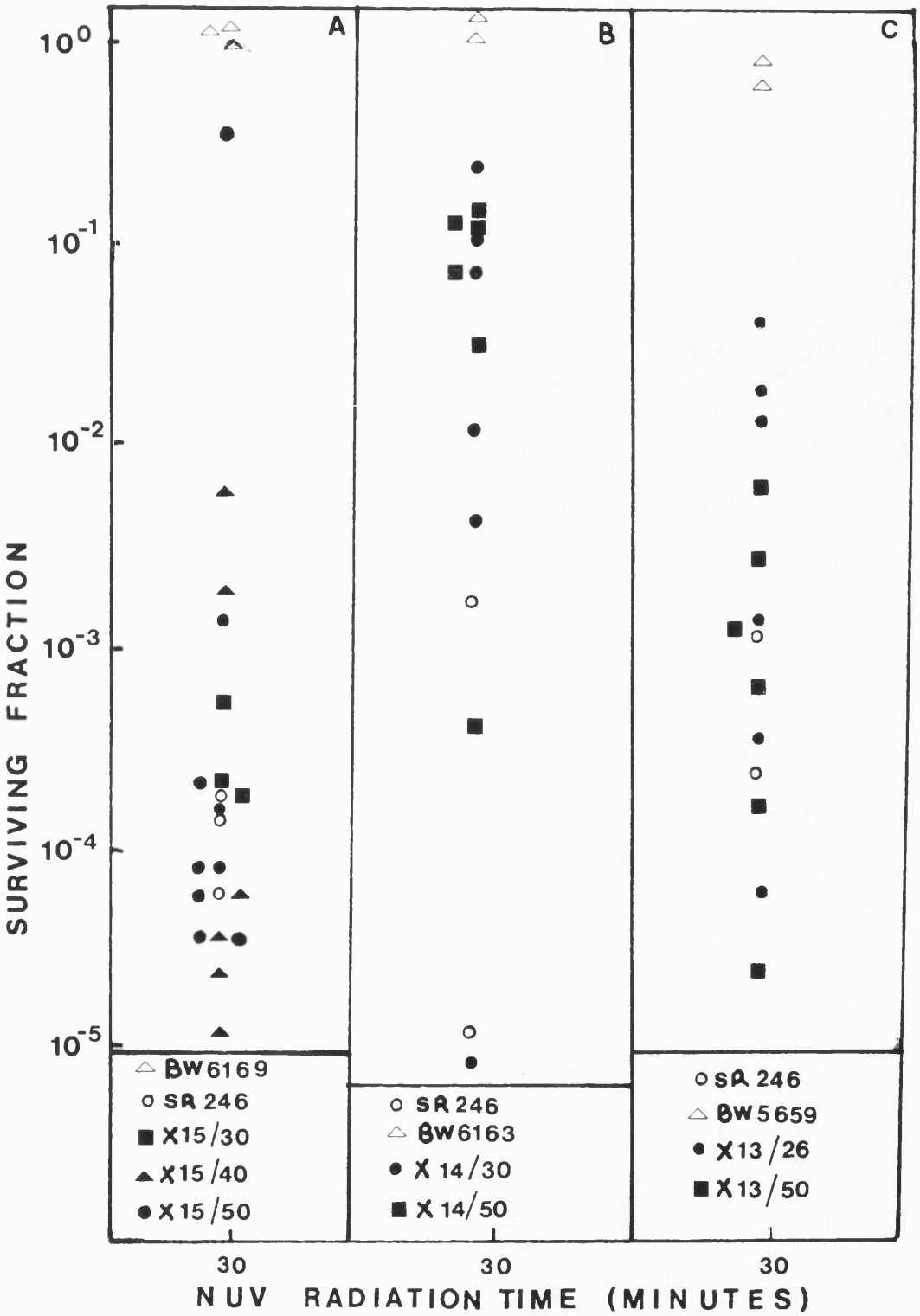


Figure 69: Surviving fractions after 30 min near-UV irradiation for *E. coli* SR246 (o), Hfr-Tn10 strains (△) and conjugants from interrupted mating experiments (A) SR246 x BW6169, (B) SR246 x BW6163, (C) SR246 x BW5659. Details for the conjugants are given in the key included in each panel.

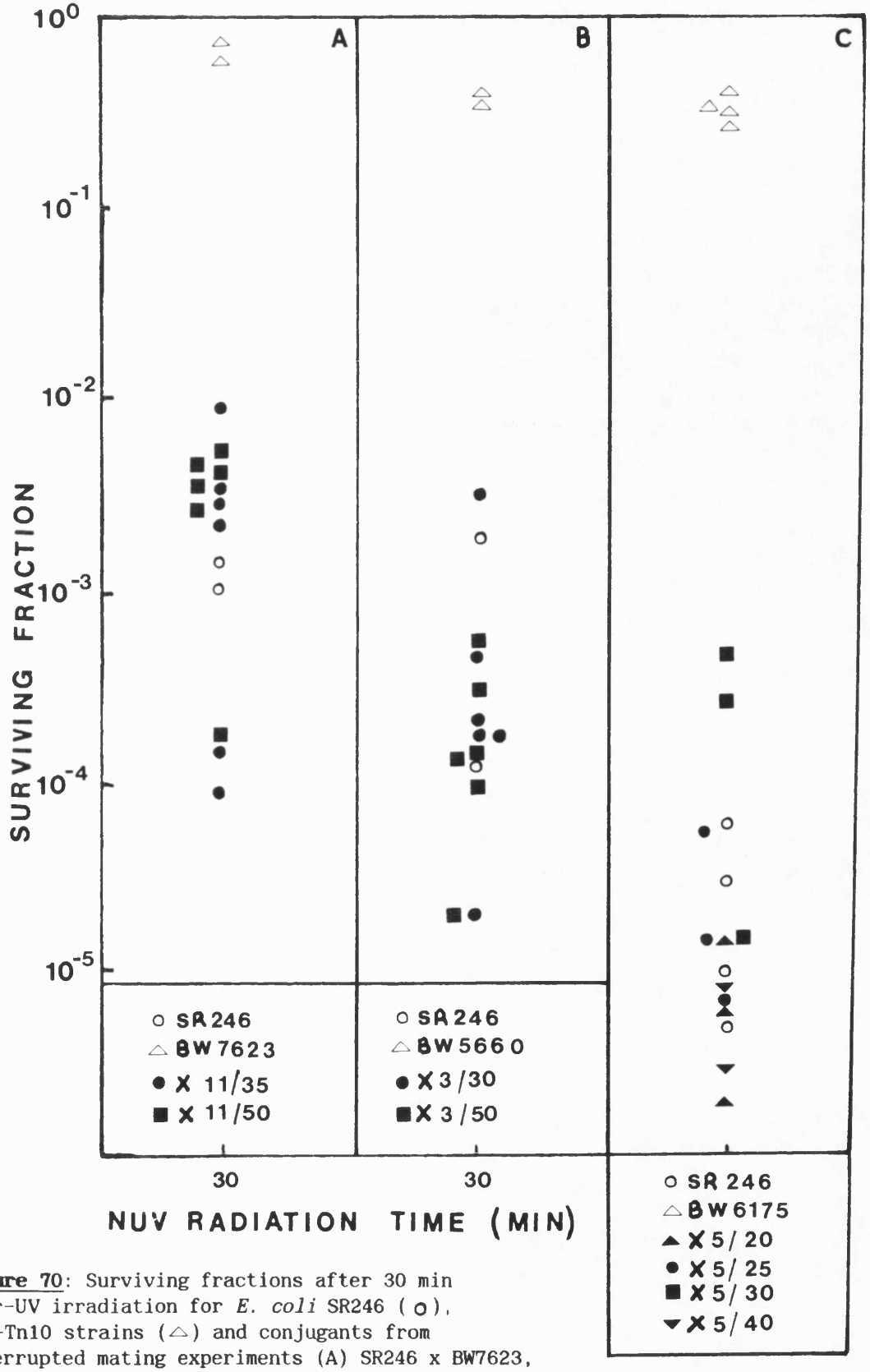


Figure 70: Surviving fractions after 30 min near-UV irradiation for *E. coli* SR246 (○), Hfr-Tn10 strains (△) and conjugants from interrupted mating experiments (A) SR246 x BW7623, (B) SR246 x BW5660, (C) SR246 x BW6175. Details for the conjugants are given in the key included in each panel.

Figure 71 shows a repeat of fig. 69B with a larger number of conjugants screened for near-UV radiation resistance. Again there is no clear point at which conjugants became radiation resistant. However, conjugants taken after 50 min conjugation tended to be more consistently near-UV radiation resistant than any of the other earlier conjugants tested. Full survival curves were performed on the three conjugants (labelled 1, 2 and 3 in fig. 71) that had greater than 10% survival. These survival curves, together with curves for SR246 and the parent Hfr-Tn10 strain, BW6163, are shown in fig. 72. The conjugants labelled 1, 2 and 3 in fig. 71 are also labelled as such in fig. 72. All three conjugants are near-UV radiation resistant. The conjugants 1 and 2 (taken 50 min after mixing) show an initial decrease in resistance which tails off at higher fluences whilst conjugant 3 (taken 35 min after mixing) shows an initial shoulder region followed by increasing sensitivity at higher fluences.

Discussion

The experiments presented in part II of this section of results were undertaken in order to try and obtain some indication of the position of the gene controlling near-UV radiation sensitivity in *E. coli* SR246. Conjugations between SR246 and a series of Hfr-Tn10 strains covering different regions of the chromosome were carried out and resultant conjugants screened for near-UV radiation sensitivity. It was expected that it would be possible to identify a clear point at which a transfer from near-UV sensitivity to resistance was seen. However, this was not the case.

Conjugants from spot conjugations (fig. 64) gave a preliminary indication as to which Hfr-Tn10 strains may have been of use. More detailed interrupted mating experiments extended the study but although

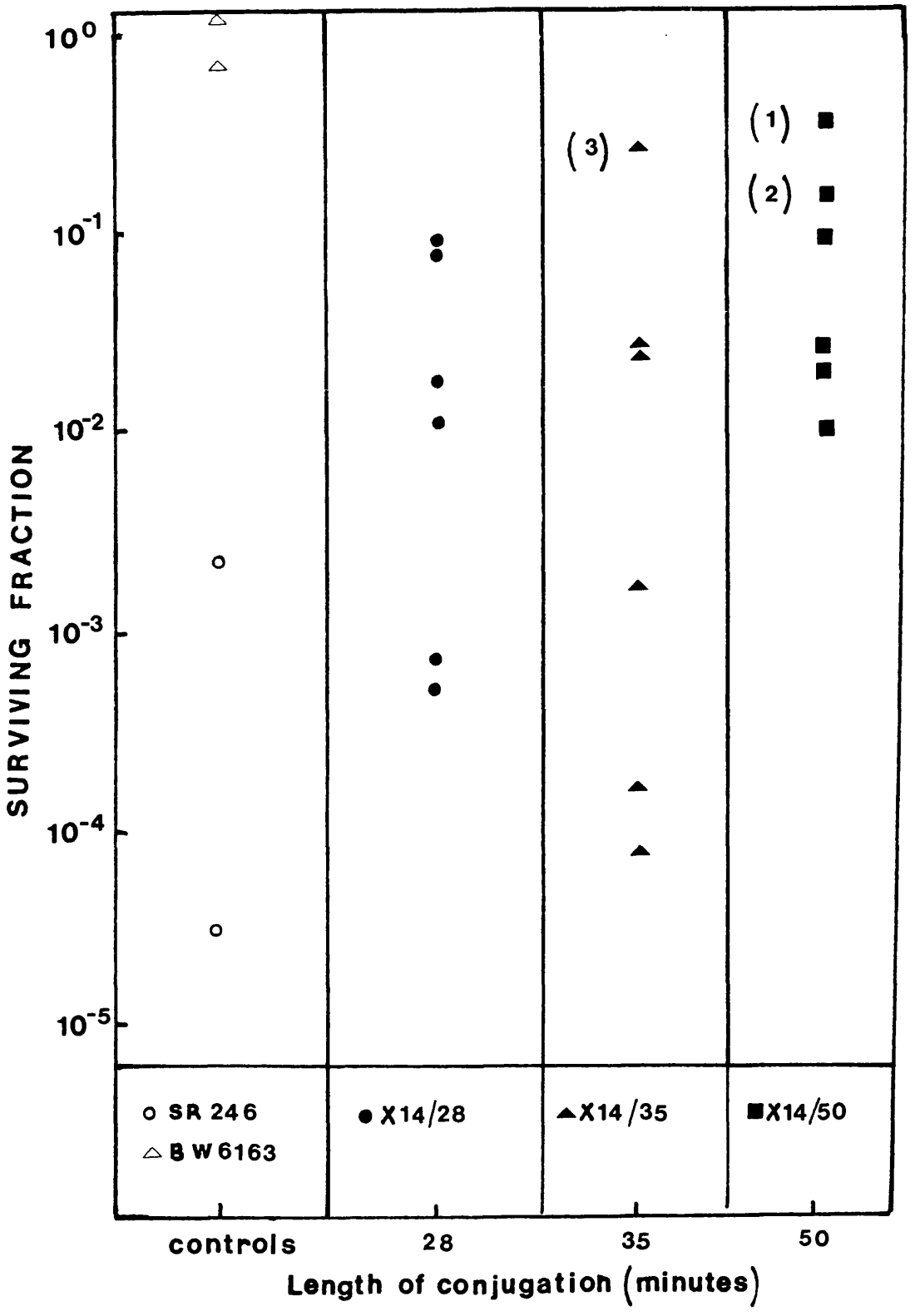


Figure 71: Surviving fractions for *E. coli* SR246 (o), BW6163 (△) and 18 X14 conjugants, after 30 min near-UV irradiation. Duration of conjugation is shown at the foot of each panel.

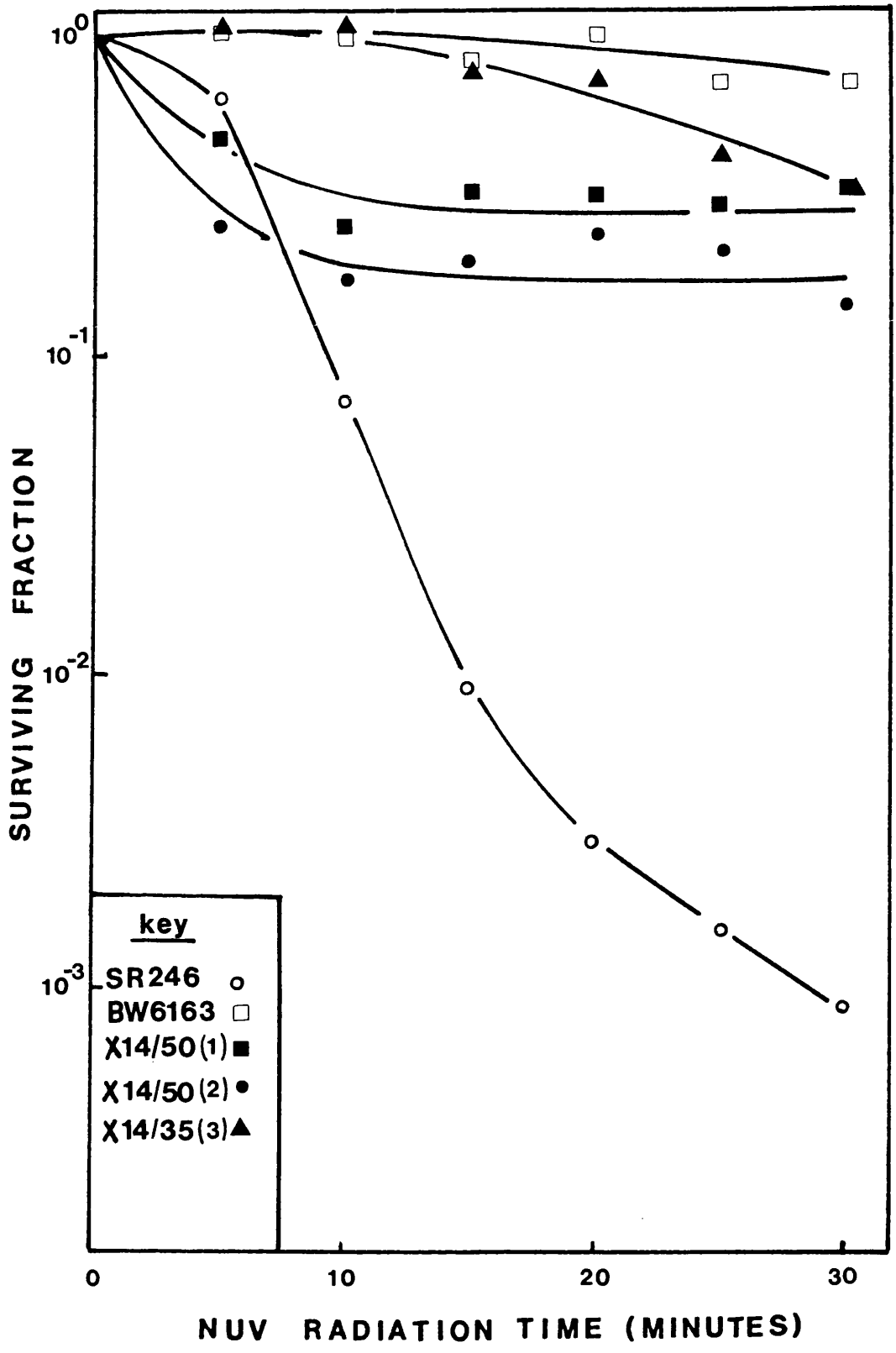


Figure 72: Survival curves for *E. coli* SR246 (o), BW6163 (□) and 3 X14 conjugants, after near-UV irradiation. Viability was assessed on YENB.

there was some degree of correlation between results (e.g. resistance seen with both spot conjugation and interrupted mating with X14 and X15 conjugants) there were also discrepancies (e.g. resistance with X9 conjugants after spot conjugation but not after interrupted mating). In some cases it is possible to argue that the results obtained indicated that the locus involved in SR246 near-UV radiation is being transferred towards the end of the Hfr-Tn10 region being studied. For example, results obtained after crossing SR246 with BW6163 and screening 12 conjugants (fig. 69B) show that a greater proportion of conjugants taken after 50 min mating show near-UV radiation resistance than do conjugants taken after 30 min mating. However, if this were so, it would be expected that a higher proportion of X13 and X11 conjugants would show near-UV radiation resistance than was actually seen (figs. 69C and 70A).

The results obtained with X3 and X5 conjugants would also indicate that the regions covered by early X15 and early X14 conjugants are not involved. X13 and X5 cover almost identical regions as X14 and X15 respectively, but in the opposite direction (see fig. 68) which reinforces the non-involvement of these shared regions.

The results obtained from the SR246 x BW6163 (X14) crosses (figs. 69B and 71) clearly show that there is some important effect in this region and the survival curves of selected conjugants (fig. 72) show the marked degree of resistance obtained. The results obtained in part I of this section of results with inactivation by rose bengal-generated singlet oxygen (fig. 54) were obtained using the X14/50 (1) conjugant, labelled in fig. 71. It is interesting to note that both resistance to near-UV radiation and singlet oxygen have been acquired in this strain. This may indicate that a gene governing some aspect of membrane function is involved.

No clear cut answer is given by the results obtained in this section into the near-UV radiation sensitivity of SR246. The results obtained in part II indicate that there is some genetic involvement. However, it is not possible to determine whether or not this is due to a single point mutation or to a more complex combination of mutations. It is also possible that the near-UV radiation sensitivity of SR246 is a combination of genetic and phenotypic responses. Further studies on SR246 could include a detailed study of membrane effects including analysis of membrane composition and leakage studies after near-UV irradiation. Further genetic experiments may also help in pinpointing the locus (loci) involved in this effect.

CONCLUDING REMARKS

The experiments presented and discussed in section 1 of the results examined the role of oxidative damage in near-UV radiation lethality in *E. coli* cells. It was shown that pretreating cells with low, non-lethal levels of H_2O_2 (30 µM) induced an increased resistance to subsequent challenge with toxic levels of H_2O_2 (5 mM). This pretreatment was also shown to induce resistance to subsequent near-UV radiation challenge. The results obtained were in agreement with published data (Sammartano and Tuveson, 1985; Tyrrell, 1985) and indicated that there was some degree of overlap between responses to near-UV radiation and H_2O_2. The results also indicated that H_2O_2 has a role in near-UV radiation damage. However, the role played by H_2O_2 in near-UV radiation damage is not straightforward. Although there was a common inducible response to both H_2O_2 and near-UV radiation, experiments showed that catalase deficient mutants sensitive to H_2O_2 were not correspondingly sensitive to near-UV radiation. In addition, one *E. coli* strain, B/r, which was not sensitive to H_2O_2, which showed a marked inducible effect and exhibited H_2O_2 scavenging ability, was shown to be very sensitive to near-UV radiation.

Exogenous catalase was seen to enhance protection against near-UV radiation damage with all strains tested and with both exponential and stationary phase cells, again in agreement with published data (Sammartano and Tuveson, 1984). This also implicates H_2O_2 in near-UV radiation damage.

A recent study by Farr *et al.* (1988) has shown that H_2O_2 challenge disrupts membrane functions in *E. coli*. Farr *et al.* (1988) have shown that H_2O_2 decreases the uptake of lactose and guanosine. The uptake of both of these compounds requires the activity of their corresponding permeases and both permease activities are in turn dependent on the electrochemical potential (or proton motive force) across the cell membrane. Inhibition of uptake was seen both with and without disruption of the proton motive

force. Farr *et al.* (1988) concluded that H_2O_2 treatment inhibits proton motive force-dependent and independent transport. They also found that the pretreatment of cells with low concentrations of H_2O_2 gave the cells the ability to recover from this inhibition of membrane transport. Addition of exogenous catalase also aided the recover of transport functions after H_2O_2 challenge. Farr *et al.* (1988) suggest that H_2O_2 oxidizes target molecule(s) in the membrane, thus disrupting membrane integrity.

The importance of membrane integrity in near-UV radiation damage has already been studied (e.g. Kelland *et al.*, 1983a, 1983b, 1984a; Chamberlain and Moss, 1987 and see general introduction). Kelland *et al.* (1983b) have shown that near-UV radiation induces sensitivity to inorganic salt and that the subsequent recovery of cells was decreased by the presence of bacitracin, an antibiotic which acts by inhibiting membrane synthesis. Furthermore, Kelland *et al.* (1984a) have also shown increased leakage of intracellular material from cells irradiated with broad-band near-UV radiation, again indicating that near-UV radiation damages the permeability barrier of the cells. The work of Farr *et al.* (1988) supports the idea that near-UV radiation induced oxidative damage could cause cell lethality via membrane damage.

Further evidence for a role for membrane damage in near-UV radiation effects comes from a report by Pizarro and Orce (1988). They reported that membrane functions, as determined by uptake of [^{14}C] alanine and the activity of the membrane bound enzyme, succinic dehydrogenase, were strongly inhibited by near-UV radiation. This inhibition occurred in conjunction with growth inhibition, a near-UV radiation sublethal effect, and with the intracellular accumulation of ppGpp and pppGpp. When cells were grown in pre-irradiation conditions that were known to alter membrane activities, the degrees of growth delay and membrane function activities

were also altered. Data presented in sections 2 and 3 of the results also showed that pre-irradiation growth conditions are important in near-UV radiation sensitivity. The results seen in section 2 with *E. coli* B/r strains indicated that near-UV radiation sensitivity was related to the stringent response, as differences were seen between isogenic *relA*$^+$ and *relA*$^-$ strains in their responses to near-UV irradiation. Pizarro and Orce (1988) found that with sublethal near-UV radiation fluences, cells recovered from near-UV radiation induced growth delay when ppGpp concentrations returned to basal levels. However, this was only true for cells that also recovered normal membrane activites.

With SR246, in section 3 of the results, it was shown that the near-UV radiation sensitivity of this strain was highly dependent on pre-irradiation growth conditions and, to a lesser extent, post-irradiation growth media. These results and those obtained after exposure of SR246 to rose bengal generated singlet oxygen again suggest that the membrane is important in near-UV radiation effects and that there is a relationship between near-UV radiation lethality, oxidative damage and the cell membrane.

In the light of these recent reports, it would be of interest to extend the experiments presented in this thesis to include a detailed and comprehensive study of membrane function and activity after near-UV radiation, H_2O_2 and other forms of oxidative challenge.

APPENDIX A

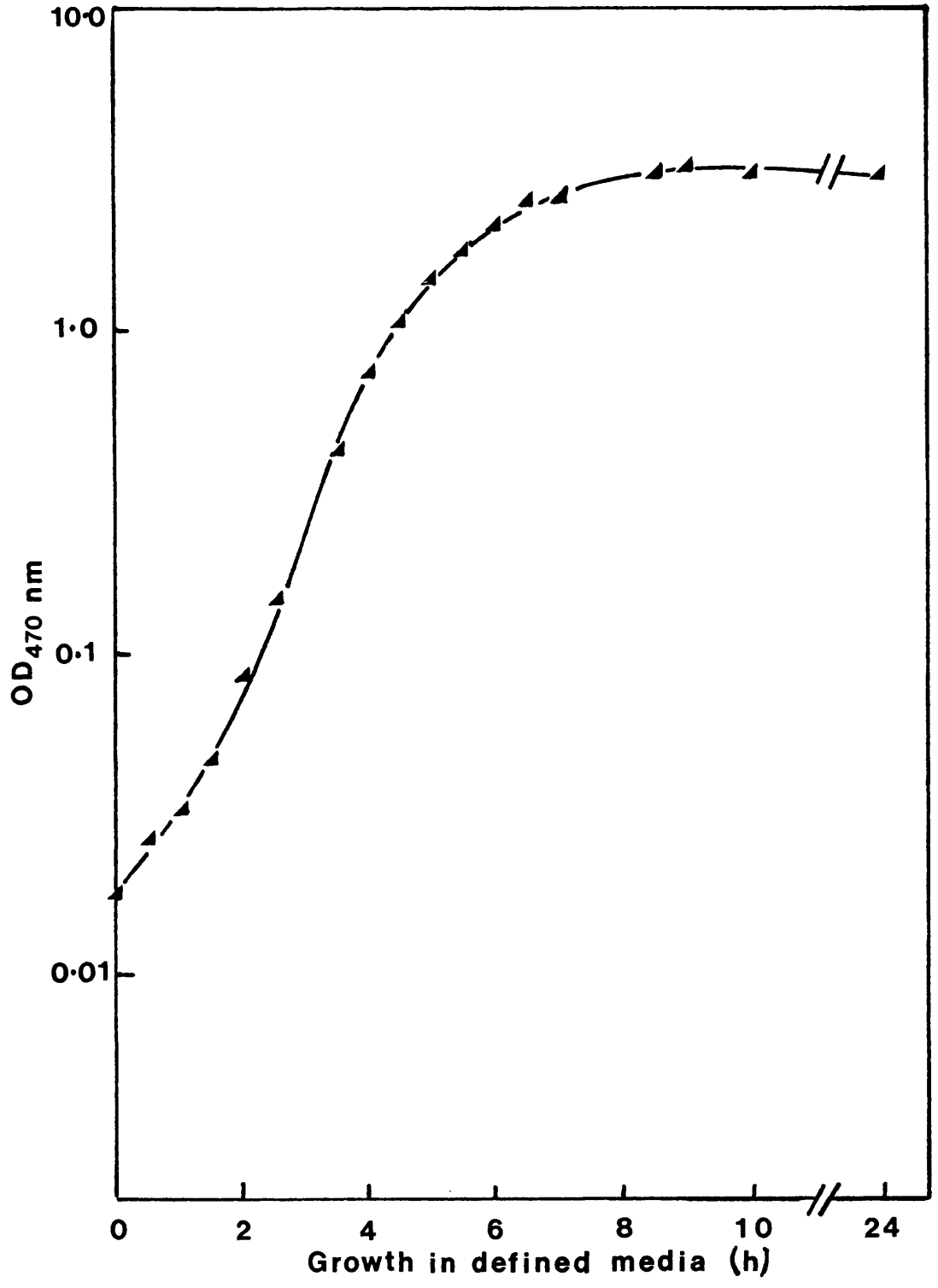


Figure 1.A: Growth curve of *E. coli* K12 AB1157 for growth in defined media.

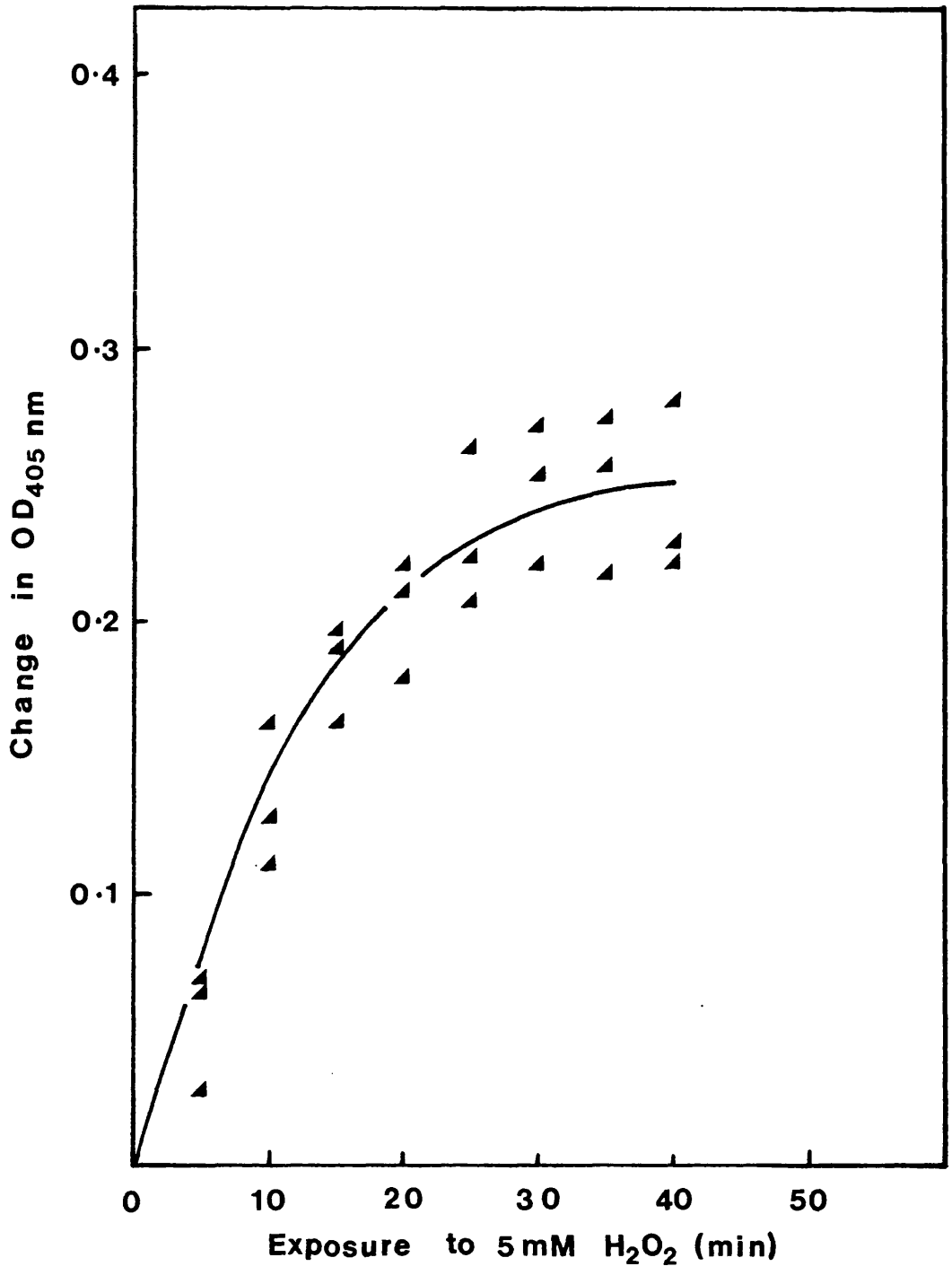


Figure 2.A: The H_2O_2 scavenging ability of exponential phase *E. coli* B/r challenged with 5 mM H_2O_2, showing the variation between replicate samples.

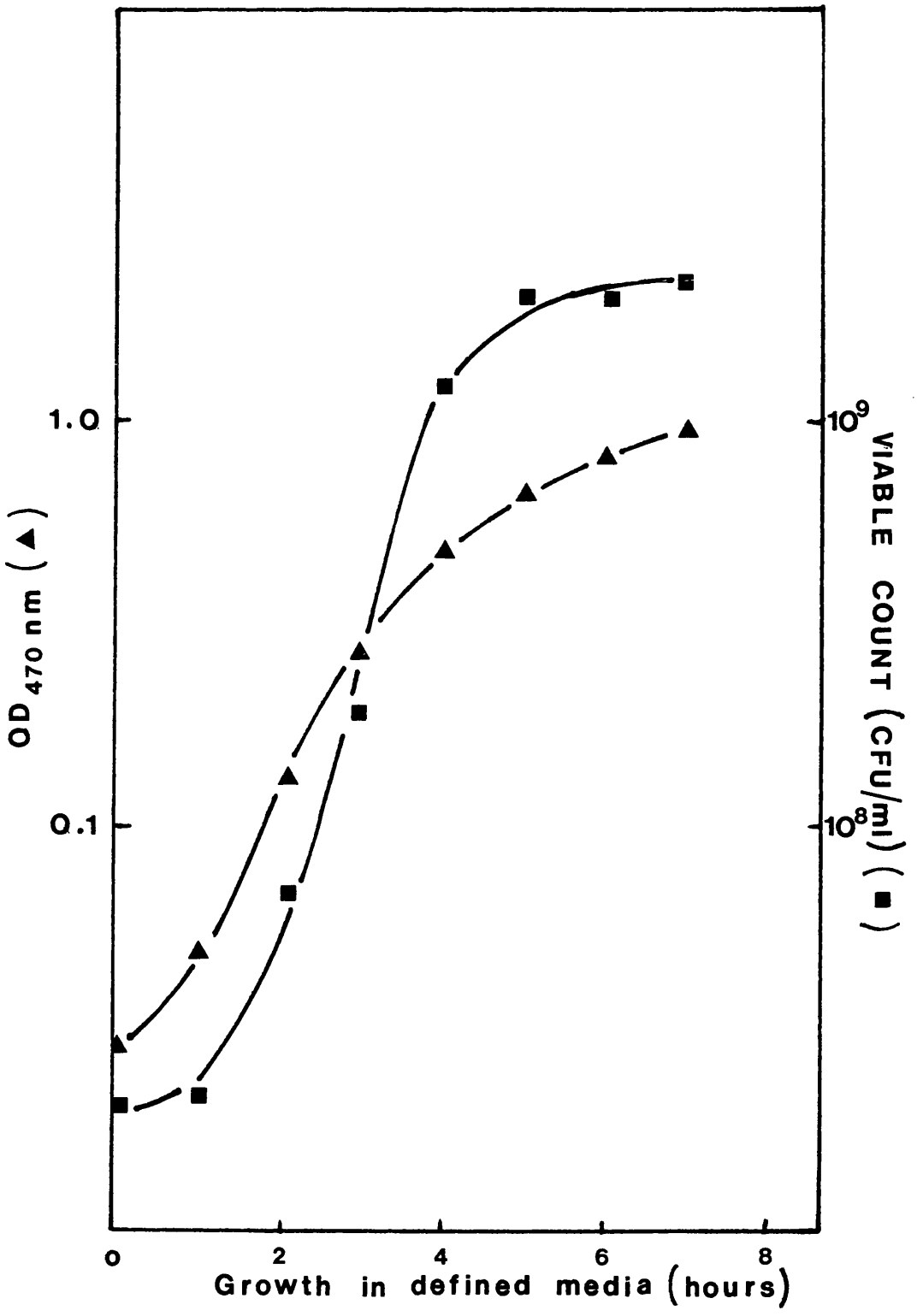


Figure 3.A: Comparison of growth curves obtained by optical density (470 nm) readings (▲) and by viable count (■) for a culture of *E. coli* B/r grown in defined media for the number of hours shown on the abscissa.

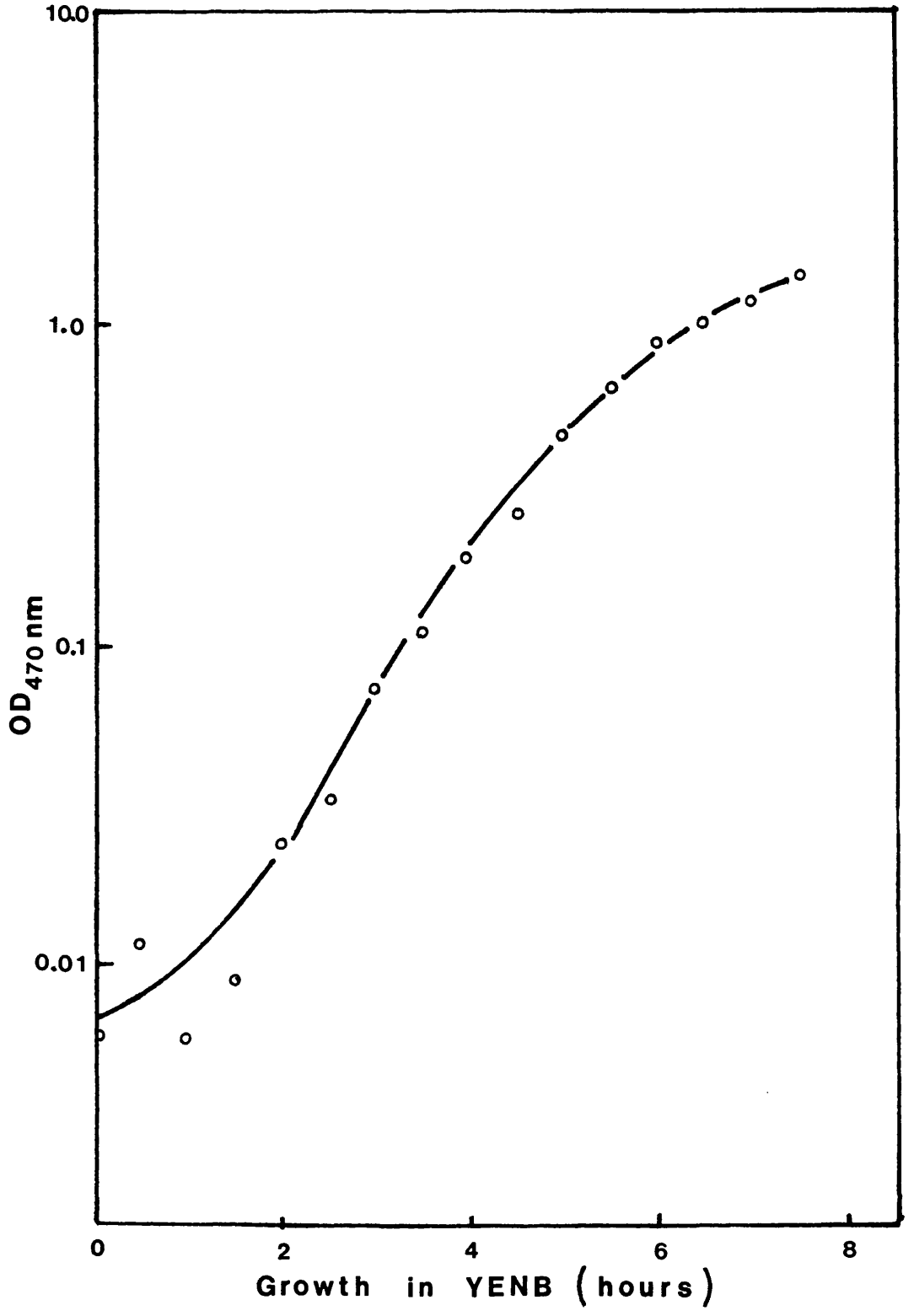


Figure 4.A: Growth curve of *E. coli* SR246 for growth in YENB.

Strain	Genotype	Parent	Construction or source
BW5659, CGSC6752	HfrKL98(KL983, PO53) *xyl-7 lacY1 mglP1 zdh-57* : : Tn*10*	KL983	Tc^R with P1 grown on K5157
BW5660, CGSC6753	HfrPK19(PK191, PO66) *thi-1 relA1*? DE5(*gpt proA proB-lac*)*supE44 spoT1*? *srlC300* : : Tn*10*	PK191	Tc^R with P1 grown on BW744
BW6156, CGSC6754	HfrP4X(KL227, BW113, NH4113, PO3) *zje* : : Tn*10 metB1 relA1 spoT1*	KL227	Tc^R with P1 grown on CAG1593
BW6159, CGSC6755	HfrKL14(PO68) *ilv-691* : : Tn*10 thi-1 relA1 spoT1*	KL14	Tc^R with P1 grown on BW646†
BW6160, CGSC6756	HfrBroda 8(PO118) *zdh-57* : : Tn*10 metB1 relA1 spoT1* λ^R	Broda 8	Tc^R with P1 grown on K5157
BW6163, CGSC6758	HfrKL16(PO45) *zed-977* : : Tn*10 thi-1 relA1 spoT1*	KL16	Tc^R with P1 grown on P2214
BW6164, CGSC6759	HfrRa-2(PO48) *thr-43* : : Tn*10 mal-28 sfa-4 supE42*	Ra-2	Tc^R with P1 grown on DB6981
BW6165, CGSC6760	HfrP801(PO120) *argE* : : Tn*10 lacY1* or *lacY40 xyl-7 mtl-2 ara-41 λind*	P801	Tc^R with P1 grown on S10
BW6166, CGSC6761	HfrJ4(KL209, P10, PO18) *zhf* : : Tn*10-721 thi-1 malB16* : : F1 (λ^R) *supE44*	KL209	Tc^R with P1 grown on SH175
BW6169, CGSC6762	HfrAB313(KL228, PO13) *argA81* : : Tn*10 thi-1 leuB6 gal-6 lacY1* or *lacZ4 supE44*	KL228	Tc^R with P1 grown on GW3210
BW6175, CGSC6763	HfrPK(PO131) *argE* : : Tn*10 thr-1 leuB6 thi-1 lacY1 azi-15 tonA21 supE44*	PK3	Tc^R with P1 grown on S10
BW7261, CGSC6787	HfrCavalli (KL226, PO2A) Δ(*argF-lac*)*205*(U169) *leu-63* : : Tn*10 relA1 tonA22 pit-10 spot1 ompF627*	BW6459	Spontaneous Pro^+ Kan^S mutant
BW7620	HfrKL99 *zed-977* : : Tn*10 thi-1 relA1 lac-42*	KL99	Tc^R with P1 grown on P2214
BW7622	HfrKL96 *trpB114* : : Tn*10 thi-1 relA1*	KL96	Tc^R with P1 grown on W3110 *trpB114*
BW7623	HfrBroda 7(KL108) *purE* : : Tn*10 relA1*?	KL208	Tc^R with P1 grown on NK6051
NK6051	HfrH(PO1) *purE* : : Tn*10* DE5 (*gpt proA proB lac*) *thi*		N. Kleckner

Table 1.A: Hfr-Tn10 mapping strains, from Wanner, 1986.

APPENDIX B

TABLE 1

Data pertaining to fig. 20

TREATMENT	TIME OF EXPOSURE TO 5mM H_2O_2 (MIN)	DILUTION	PLATE COUNT	MEAN PLATE COUNT	VIABLE COUNT (ml^{-1})	SURVIVING FRACTION
UNPRETREATED	0	10^5	401, 379, 327	369	3.69×10^7	1.0
	10	10^5	349, 311, 334	331	3.31×10^7	9.00×10^{-1}
	20	10^5	226, 246, 241	238	2.38×10^7	6.40×10^{-1}
	30	10^5	145, 199, 174	173	1.73×10^7	4.70×10^{-1}
	40	10^5	121, 93, 91	102	1.02×10^7	1.02×10^{-1}
	60	10^4	55, 58, 58	57	5.70×10^5	1.50×10^{-2}
	80	10^2	81, 117, 81	93	9.30×10^3	2.50×10^{-4}
PRETREATED	0	10^5	347, 414, 352	371	3.71×10^7	1.0
	10	10^5	263, 326, 250	280	2.80×10^7	7.50×10^{-1}
	20	10^5	270, 236, 281	262	2.62×10^7	7.10×10^{-1}
	30	10^5	228, 280, 198	235	2.35×10^7	6.30×10^{-1}
	40	10^5	177, 140, 158	158	1.58×10^7	4.30×10^{-1}
	60	10^5	105, 72, 105	94	9.40×10^6	2.50×10^{-1}
	80	10^5	61, 61, 75	66	6.60×10^6	1.80×10^{-1}

continued/...

Table 1 continued

Data for replicate experiment

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	SURVIVING FRACTION	
	UNPRETREATED.	PRETREATED.
0	1.0	1.0
10	7.70×10^{-1}	9.70×10^{-1}
20	6.90×10^{-1}	7.40×10^{-1}
30	4.60×10^{-1}	4.50×10^{-1}
40	4.00×10^{-2}	4.30×10^{-1}
60	2.60×10^{-3}	2.20×10^{-1}
80	1.00×10^{-4}	9.20×10^{-2}

TABLE 2

Data pertraining to fig. 21 A and B

FIGURE 21 A: *E coli* B/r

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	SURVIVING FRACTION	
	UNPRETREATED	PRETREATED
0	1.0	1.0
20	1.94×10^{-1}	4.94×10^{-1}
40	7.90×10^{-3}	3.89×10^{-1}
60	2.40×10^{-4}	2.87×10^{-1}
80	-	2.39×10^{-1}

Replicate experiment

0	1.0	1.0
20	2.60×10^{-1}	8.40×10^{-1}
40	2.30×10^{-2}	4.80×10^{-1}
60	1.80×10^{-3}	3.20×10^{-1}
80	2.00×10^{-4}	1.24×10^{-1}

FIGURE 21 B: *E coli* K12 SR385

0	1.0	1.0
10	3.18×10^{-1}	7.69×10^{-1}
20	1.56×10^{-1}	8.65×10^{-1}
30	1.00×10^{-1}	4.52×10^{-1}
40	2.16×10^{-2}	1.25×10^{-1}
50	1.38×10^{-3}	9.04×10^{-3}
60	2.00×10^{-4}	3.80×10^{-3}
80	-	2.00×10^{-3}

Replicate experiment

0	1.0	1.0
20	2.51×10^{-1}	7.63×10^{-1}
40	1.85×10^{-2}	3.00×10^{-1}
60	6.60×10^{-4}	8.30×10^{-3}
80	2.60×10^{-5}	1.80×10^{-3}

TABLE 3

Data pertaining to fig. 22 A and B

FIGURE 22A: *E coli* UMI

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	SURVIVING FRACTION UNPRETREATED	PRETREATED
0	1.0	1.0
10	6.88×10^{-1}	7.11×10^{-1}
20	4.80×10^{-1}	1.27×10^{-1}
30	1.96×10^{-1}	1.50×10^{-2}
40	1.05×10^{-2}	1.70×10^{-3}
50	2.40×10^{-4}	1.90×10^{-4}
60	3.80×10^{-5}	6.00×10^{-5}
80	-	-

Replicate experiment

0	1.0	1.0
10	4.60×10^{-1}	5.58×10^{-1}
20	1.44×10^{-1}	2.50×10^{-1}
30	-	6.50×10^{-2}
40	3.30×10^{-3}	6.70×10^{-3}
50	4.70×10^{-4}	5.90×10^{-4}
60	2.90×10^{-4}	5.30×10^{-5}
80	1.50×10^{-5}	3.70×10^{-5}

FIGURE 22B: *E coli* CSH7

0	1.0	1.0
10	5.86×10^{-1}	5.03×10^{-1}
20	2.78×10^{-1}	2.60×10^{-1}
30	1.32×10^{-1}	1.10×10^{-1}
40	5.70×10^{-2}	7.70×10^{-2}
50	9.20×10^{-3}	5.90×10^{-2}
60	2.60×10^{-3}	4.40×10^{-2}
80	1.00×10^{-4}	1.67×10^{-2}

Replicate experiments

0	1.0	1.0
10	3.05×10^{-1}	4.56×10^{-1}
20	2.44×10^{-1}	1.57×10^{-1}
30	4.09×10^{-2}	6.70×10^{-2}
40	2.00×10^{-3}	3.90×10^{-2}
50	1.30×10^{-4}	1.80×10^{-2}
60	7.00×10^{-5}	8.00×10^{-3}
80	-	7.80×10^{-4}

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	SURVIVING FRACTION	
	UNPRETREATED	PRETREATED
0	1.0	1.0
10	7.04×10^{-1}	4.78×10^{-1}
20	3.94×10^{-1}	3.06×10^{-1}
30	1.64×10^{-1}	1.26×10^{-1}
40	8.75×10^{-2}	9.19×10^{-2}
50	2.92×10^{-2}	5.65×10^{-2}
60	5.67×10^{-3}	3.29×10^{-2}
80	9.00×10^{-5}	2.50×10^{-3}

TABLE 4

Data pertaining to fig. 23 A and B

FIGURE 23A: *E coli* K12 AB1157

TIME OF IRRADIATION (min)	SURVIVING FRACTION	
	UNPRETREATED	PRETREATED
0	1.0	1.0
20	1.13	1.16
40	5.83×10^{-1}	8.38×10^{-1}
60	2.70×10^{-1}	5.13×10^{-1}
80	1.37×10^{-3}	1.05×10^{-2}
100	1.50×10^{-4}	1.60×10^{-4}
120	3.40×10^{-5}	1.80×10^{-4}

Replicate experiment

0	1.0	1.0
20	7.20×10^{-1}	8.40×10^{-1}
40	4.40×10^{-1}	8.20×10^{-1}
60	1.30×10^{-1}	2.60×10^{-1}
80	3.80×10^{-3}	5.00×10^{-3}
100	1.50×10^{-4}	3.00×10^{-4}
120	2.00×10^{-5}	6.00×10^{-5}

FIGURE 23B: *E coli* B/r

0	1.0	1.0
20	5.00×10^{-3}	1.16
40	7.00×10^{-4}	6.54×10^{-1}
60	1.90×10^{-4}	4.67×10^{-1}
80	6.00×10^{-5}	1.40×10^{-1}
100	1.00×10^{-5}	2.00×10^{-2}

Replicate experiment

0	1.0	1.0
20	2.2×10^{-4}	7.53×10^{-1}
40	5.5×10^{-5}	4.20×10^{-1}
50	7.6×10^{-5}	3.50×10^{-1}
60	5.7×10^{-5}	1.90×10^{-1}
80	2.7×10^{-5}	2.05×10^{-2}

TABLE 5

Data pertaining to fig. 24: *E coli* SR385

TIME OF IRRADIATION (min)	SURVIVING FRACTION	
	UNPRETREATED	PRETREATED
0	1.0	1.0
10	9.70×10^{-1}	8.80×10^{-1}
20	6.70×10^{-1}	8.90×10^{-1}
40	2.06×10^{-1}	7.30×10^{-1}
60	1.60×10^{-2}	1.56×10^{-1}
80	1.60×10^{-3}	5.60×10^{-3}
100	3.90×10^{-4}	8.60×10^{-4}
120	4.90×10^{-4}	5.70×10^{-4}
Replicate experiment		
0	1.0	1.0
20	1.24×10^{-1}	8.72×10^{-1}
40	2.10×10^{-1}	6.68×10^{-1}
50	6.60×10^{-5}	3.25×10^{-1}
60	-	9.60×10^{-2}
80	-	9.60×10^{-4}
100	-	1.90×10^{-4}

TABLE 6

Data pertaining to fig. 25 A and B

FIGURE 25A: *E coli* UMI

TIME OF IRRADIATION (min)	SURVIVING FRACTION	
	UNPRETREATED	PRETREATED
0	1.0	1.0
10	7.83×10^{-1}	7.17×10^{-1}
20	7.39×10^{-1}	7.17×10^{-1}
30	6.30×10^{-1}	8.00×10^{-1}
40	7.17×10^{-1}	6.00×10^{-1}
50	6.02×10^{-1}	3.13×10^{-1}
60	4.52×10^{-1}	2.68×10^{-1}
80	1.09×10^{-1}	7.00×10^{-2}
100	4.10×10^{-3}	7.80×10^{-3}

Replicate experiment

0	1.0	1.0
20	8.00×10^{-1}	7.60×10^{-1}
40	6.50×10^{-1}	7.60×10^{-1}
60	4.60×10^{-1}	2.80×10^{-1}
80	1.36×10^{-1}	3.60×10^{-1}
100	8.60×10^{-3}	2.00×10^{-3}
120	2.20×10^{-3}	4.00×10^{-4}

FIGURE 25B: *E coli* CSH7

0	1.0	1.0
20	4.60×10^{-1}	8.70×10^{-1}
40	1.10×10^{-1}	7.00×10^{-1}
70	6.70×10^{-2}	5.60×10^{-1}
80	8.70×10^{-3}	5.60×10^{-1}
100	5.20×10^{-3}	1.40×10^{-1}
120	1.60×10^{-3}	4.90×10^{-2}

Replicate experiment

0	1.0	1.0
20	4.90×10^{-1}	9.20×10^{-1}
40	4.10×10^{-2}	8.00×10^{-1}
60	1.13×10^{-2}	1.90×10^{-1}
80	1.80×10^{-3}	8.80×10^{-2}
100	1.90×10^{-4}	4.70×10^{-3}
120	1.70×10^{-4}	2.70×10^{-3}

TABLE 7

Data pertaining to fig. 26 A and B

FIGURE 26A: *E coli* AB1157

TIME OF IRRADIATION (min)	SURVIVING FRACTION			
	UNPRETREATED	UNPRETREATED + CATALASE	PRETREATED	PRETREATED + CATALASE
0	1.0	1.0	1.0	1.0
20	1.56×10^{-1}	4.31×10^{-1}	5.97×10^{-1}	6.48×10^{-1}
40	6.60×10^{-2}	1.80×10^{-1}	4.49×10^{-1}	5.17×10^{-1}
50	2.40×10^{-2}	1.29×10^{-1}	2.29×10^{-1}	4.64×10^{-1}
60	8.50×10^{-2}	4.00×10^{-2}	8.60×10^{-2}	1.60×10^{-1}
80	9.60×10^{-4}	2.40×10^{-3}	1.20×10^{-3}	4.20×10^{-3}
100	5.00×10^{-5}	2.00×10^{-4}	1.20×10^{-4}	4.70×10^{-4}

Replicate experiment

0	1.0	1.0	1.0	1.0
20	3.5×10^{-1}	6.50×10^{-1}	7.20×10^{-1}	9.90×10^{-1}
40	2.00×10^{-1}	4.00×10^{-1}	6.30×10^{-1}	6.50×10^{-1}
50	1.30×10^{-1}	3.60×10^{-1}	4.70×10^{-1}	6.60×10^{-1}
60	6.04×10^{-2}	2.70×10^{-1}	1.20×10^{-1}	3.00×10^{-1}
80	2.00×10^{-3}	9.00×10^{-3}	4.40×10^{-3}	1.02×10^{-2}
100	3.80×10^{-4}	1.20×10^{-3}	9.00×10^{-4}	2.50×10^{-3}

FIGURE 26B: *E. coli* B/r

0	1.0	1.0	1.0	1.0
20	2.20×10^{-4}	8.54×10^{-1}	7.53×10^{-1}	8.09×10^{-1}
40	5.50×10^{-5}	7.54×10^{-1}	4.20×10^{-1}	5.52×10^{-1}
50	7.60×10^{-5}	5.90×10^{-1}	3.50×10^{-1}	4.81×10^{-1}
60	5.70×10^{-5}	5.03×10^{-1}	1.90×10^{-1}	4.16×10^{-1}
80	2.70×10^{-5}	3.87×10^{-2}	2.05×10^{-2}	1.37×10^{-1}
100	-	-	5.10×10^{-4}	6.10×10^{-3}

Replicate experiment

0	1.0	1.0	1.0	1.0
10	6.80×10^{-1}	-	-	-
20	5.56×10^{-2}	7.20×10^{-1}	8.90×10^{-1}	9.90×10^{-1}
40	4.30×10^{-3}	6.40×10^{-1}	8.10×10^{-1}	9.60×10^{-1}
60	4.60×10^{-4}	5.60×10^{-1}	6.80×10^{-1}	7.80×10^{-1}
80	6.60×10^{-4}	6.54×10^{-2}	8.40×10^{-2}	3.10×10^{-1}
100	3.70×10^{-4}	5.77×10^{-2}	2.20×10^{-2}	1.47×10^{-2}

TABLE 8

Data pertaining to figs. 27 and 28

FIGURE 27: *E coli* SR385

TIME OF IRRADIATION (min)	SURVIVING FRACTION			
	UNPRETREATED	UNPRETREATED + CATALASE	PRETREATED	PRETREATED + CATALASE
0	1.0	1.0	1.0	1.0
10	1.84×10^{-1}	1.02	9.04×10^{-1}	1.04
20	9.00×10^{-2}	1.02	8.27×10^{-1}	9.81×10^{-1}
30	3.60×10^{-2}	1.0	7.31×10^{-1}	1.04
40	4.90×10^{-3}	8.42×10^{-1}	3.46×10^{-1}	7.38×10^{-1}
50	5.70×10^{-4}	7.18×10^{-1}	1.27×10^{-1}	4.74×10^{-1}
60	1.10×10^{-4}	6.76×10^{-1}	3.80×10^{-3}	2.23×10^{-1}
80	1.90×10^{-4}	4.26×10^{-1}	6.10×10^{-4}	2.47×10^{-2}
100	2.30×10^{-4}	1.36×10^{-2}	1.20×10^{-4}	2.11×10^{-3}

Replicate experiment

0	1.0	1.0	1.0	1.0
10	2.05×10^{-1}	8.73×10^{-1}	1.25×10^{-1}	6.31×10^{-1}
20	2.70×10^{-2}	7.59×10^{-1}	8.33×10^{-2}	1.58×10^{-1}
30	3.35×10^{-3}	6.26×10^{-1}	4.17×10^{-2}	1.05×10^{-1}
40	8.80×10^{-4}	3.32×10^{-1}	8.33×10^{-3}	5.79×10^{-2}
50	4.80×10^{-4}	1.27×10^{-1}	4.17×10^{-3}	2.63×10^{-2}
60	2.90×10^{-4}	2.95×10^{-2}	-	2.68×10^{-3}
80	7.40×10^{-5}	3.60×10^{-3}	4.20×10^{-5}	-
100	2.90×10^{-5}	-	-	-

FIGURE 28

TIME OF IRRADIATION (min)	SURVIVING FRACTION		
	NO SOD	SOD 10µg/ml	SOD 100µg/ml
0	1.0	1.0	1.0
20	8.29×10^{-1}	1.25	1.0
40	4.66×10^{-1}	5.00×10^{-1}	5.68×10^{-1}
60	3.34×10^{-2}	7.20×10^{-2}	1.03×10^{-1}
80	2.10×10^{-4}	8.00×10^{-4}	2.59×10^{-3}

TABLE 9

Data pertaining to fig. 29 A and B

FIGURE 29A: *E coli* UMI

TIME OF IRRADIATION (min)	SURVIVING FRACTION			
	UNPRETREATED	UNPRETREATED + CATALASE	PRETREATED	PRETREATED + CATALASE
0	1.0	1.0	1.0	1.0
20	8.00×10^{-1}	7.60×10^{-1}	7.60×10^{-1}	9.20×10^{-1}
40	6.50×10^{-1}	7.50×10^{-1}	7.60×10^{-1}	6.60×10^{-1}
60	4.60×10^{-1}	7.30×10^{-1}	2.80×10^{-1}	4.20×10^{-1}
80	1.36×10^{-2}	4.40×10^{-1}	3.60×10^{-2}	1.20×10^{-1}
100	8.60×10^{-3}	-	2.00×10^{-3}	1.40×10^{-2}
120	2.20×10^{-3}	3.20×10^{-2}	4.00×10^{-4}	1.00×10^{-3}

Replicate experiment

0	1.0	1.0	1.0	1.0
20	1.0	9.50×10^{-1}	9.70×10^{-1}	9.20×10^{-1}
40	2.80×10^{-1}	7.50×10^{-1}	3.40×10^{-1}	5.60×10^{-1}
60	4.50×10^{-2}	6.50×10^{-1}	3.30×10^{-2}	2.37×10^{-1}
80	7.50×10^{-3}	8.07×10^{-2}	6.10×10^{-3}	1.60×10^{-2}
100	2.00×10^{-4}	6.60×10^{-3}	2.60×10^{-4}	3.30×10^{-3}

FIGURE 29B: *E. coli* CSH7

0	1.0	1.0
20	7.20×10^{-1}	1.04
40	2.70×10^{-1}	9.70×10^{-1}
60	7.10×10^{-2}	7.50×10^{-1}
80	5.00×10^{-2}	5.70×10^{-1}
100	-	3.90×10^{-1}
120	5.00×10^{-4}	8.00×10^{-2}

Replicate experiment

0	1.0	1.0
20	9.30×10^{-1}	9.90×10^{-1}
40	3.90×10^{-1}	9.20×10^{-1}
60	3.60×10^{-2}	7.90×10^{-1}
80	7.80×10^{-3}	1.47×10^{-1}
100	5.50×10^{-3}	6.70×10^{-2}
120	6.20×10^{-4}	1.20×10^{-2}

TABLE 10

Data pertaining to fig. 30

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	MEAN CHANGE IN OPTICAL DENSITY AT 405 nm					
	E. coli K12 AB1157		*E. coli* B/r		*E. coli* UMI	
	UNPRETREATED	PRETREATED	UNPRETREATED	PRETREATED	UNPRETREATED	PRETREATED
0	0	0	0	0	0	0
5	0.060	0.217	0.053	0.157	0	-0.020
10	0.075	0.405	0.090	0.322	0.008	0
15	0.112	0.520	0.128	0.450	0	-0.008
20	0.195	0.573	0.188	0.547	0	-0.008
25	0.232	0.663	0.225	0.607	0	-0.015
30	0.232	0.727	0.263	0.682	0.008	-0.015
40	0.195	-	0.293	0.765	0.015	-0.015
50	0.279	0.768	0.345	0.807	0.023	0.015
60	0.240	0.786	0.383	0.834	-0.007	-0.022

Initial OD_{405} = 0.32 (AB1157 & B/r); = 0.22 (UMI)

Replicate experiments

0	0	0	0	0	0	0
5	-	0.183	0.060	0.090	-	-
10	-0.044	0.309	0.036	0.135	0.005	0
15	0.066	0.306	0.123	0.203	-	-
20	0.042	0.324	0.126	0.255	0.007	0
25	0.042	0.282	-	-	-	-
30	0.003	0.297	0.144	0.225	0.070	-0.005
40	-0.069	0.267	0.180	0.360	0.040	-0.015
50	-	-	-	-	-0.040	0.030
60	-	-	0.219	0.405	0.010	-0.040

Initial OD_{405} = 0.28 (AB1157); = 0.15 (B/r); = 0.22 (UMI)

TABLE 11

Data pertaining to figs. 31 and 32

FIGURE 31

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	MEAN CHANGE IN OPTICAL DENSITY AT 405 nm			
	E. coli UMI		*E. coli* CSH7	
	UNPRETREATED	PRETREATED	UNPRETREATED	PRETREATED
0	0	0	0	0
10	0.005	0	0.200	0.289
20	0.007	0	0.292	0.378
30	0.070	-0.005	0.333	0.486
40	0.040	-0.015	0.375	0.520
50	-0.040	0.030	-	-
60	0.010	-0.040	-	-

Initial OD_{405} = 0.22 (UMI)
= 0.22 (CSH7)

Replicate experiment

0	0	0
10	0.008	0
20	0	-0.008
30	0.008	-0.015
40	0.015	-0.015
50	0.023	0.015
60	-0.007	-0.022

Initial OD_{405} = 0.22 (UMI)

FIGURE 32

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	MEAN CHANGE IN OPTICAL DENSITY AT 405 nm	
	UNPRETREATED	PRETREATED
0	0	0
10	-0.018	0.042
20	-0.012	0.135
30	-0.015	0.144
40	-	0.105
50	0.012	0.177
60	0	0.183
70	-0.018	0.168

TABLE 12

Data pertaining to fig. 33 A and B

FIGURE 33A: (H_2O_2 challenge)

TIME OF EXPOSURE TO 5mM H_2O_2 (min)	SURVIVING FRACTION	
	UNPRETREATED	PRETREATED
0	1.0	1.0
20	6.40×10^{-1}	8.30×10^{-1}
40	1.40×10^{-1}	6.50×10^{-1}
60	1.30×10^{-2}	2.90×10^{-1}
80	-	1.30×10^{-1}
100	-	1.60×10^{-2}
120	-	3.00×10^{-4}

Replicate experiment

0	1.0	1.0
20	6.79×10^{-1}	4.21×10^{-1}
40	3.40×10^{-1}	4.34×10^{-1}
60	1.70×10^{-2}	2.68×10^{-1}
80	2.00×10^{-3}	4.60×10^{-2}
100	-	9.00×10^{-3}
120	-	2.00×10^{-3}

FIGURE 33B (near-UV challenge)

TIME OF IRRADIATION		
0	1.0	1.0
20	8.80×10^{-1}	8.40×10^{-1}
40	1.06	4.60×10^{-1}
60	8.80×10^{-1}	8.00×10^{-2}
80	7.10×10^{-1}	5.00×10^{-4}
100	6.60×10^{-1}	-
120	4.70×10^{-1}	-

Replicate experiment

TIME OF IRRADIATION		
0	1.0	1.0
20	1.0	3.90×10^{-1}
40	4.40×10^{-1}	2.60×10^{-2}
60	4.10×10^{-1}	2.05×10^{-3}
80	2.20×10^{-1}	1.40×10^{-4}
100	1.80×10^{-1}	8.00×10^{-5}
120	-	2.50×10^{-5}

TABLE 13

Data pertaining to figs. 34 A and B

FIGURE 34A: *E. coli* K12 AB1157

TIME OF IRRADIATION (min)	SURVIVING FRACTION WITHOUT CATALASE	WITH CATALASE
0	1.0	1.0
20	8.17×10^{-1}	-
25	-	8.40×10^{-1}
40	4.61×10^{-1}	5.57×10^{-1}
60	7.10×10^{-2}	1.41×10^{-1}
80	7.40×10^{-3}	3.20×10^{-2}
100	3.10×10^{-4}	2.20×10^{-3}

Replicate experiment

0	1.0	1.0
20	7.00×10^{-1}	7.07×10^{-1}
40	3.69×10^{-1}	4.23×10^{-1}
60	1.75×10^{-1}	2.68×10^{-1}
80	4.90×10^{-2}	1.45×10^{-1}
100	3.40×10^{-3}	2.69×10^{-2}

FIGURE 34B: *E. coli* B/r

0	1.0	1.0
10	5.71×10^{-1}	7.05×10^{-1}
20	4.70×10^{-1}	7.21×10^{-1}
40	4.41×10^{-1}	6.07×10^{-1}
60	3.07×10^{-1}	4.28×10^{-1}
80	2.41×10^{-1}	3.18×10^{-1}
100	1.61×10^{-1}	1.13×10^{-1}

Replicate experiment

0	1.0	1.0
10	8.29×10^{-1}	1.08
20	9.23×10^{-1}	8.32×10^{-1}
40	4.19×10^{-1}	7.79×10^{-1}
60	1.28×10^{-1}	5.31×10^{-1}
80	4.27×10^{-2}	1.50×10^{-1}
100	1.37×10^{-2}	3.18×10^{-1}

TABLE 14

Data pertaining to figs. 35 A and B

FIGURE 35A: *E. coli* SR 385

TIME OF IRRADIATION (min)	SURVIVING FRACTION WITHOUT CATALASE	WITH CATALASE
0	1.0	1.0
20	5.74×10^{-1}	5.93×10^{-1}
30	1.40×10^{-1}	4.92×10^{-1}
40	1.75×10^{-2}	4.07×10^{-1}
60	1.10×10^{-4}	1.23×10^{-1}
80	-	2.12×10^{-2}

Replicate experiment

0	1.0	1.0
20	9.50×10^{-1}	1.20
40	4.40×10^{-1}	1.35
60	3.50×10^{-1}	1.00
80	8.50×10^{-2}	8.28×10^{-1}
100	1.00×10^{-3}	1.84×10^{-1}

FIGURE 35B: *E. coli* UMI

0	1.0	1.0
20	9.40×10^{-1}	8.70×10^{-1}
40	5.80×10^{-1}	6.20×10^{-1}
60	5.30×10^{-1}	6.40×10^{-1}
80	3.80×10^{-1}	5.50×10^{-1}
100	1.20×10^{-1}	3.30×10^{-1}
120	8.00×10^{-2}	2.50×10^{-1}

Replicate experiment

0	1.0	1.0
20	7.00×10^{-1}	7.28×10^{-1}
40	7.40×10^{-1}	7.28×10^{-1}
60	3.70×10^{-1}	5.44×10^{-1}
80	1.20×10^{-1}	3.01×10^{-1}
100	4.40×10^{-2}	1.65×10^{-1}
120	4.40×10^{-3}	5.34×10^{-2}

TABLE 15

Data pertaining to fig. 36

TIME OF IRRADIATION (min)	SURVIVING FRACTION *E. coli* B/r	*E. coli* K12 AB1157
0	1.0	1.0
20	7.40×10^{-1}	1.13
40	8.60×10^{-1}	8.80×10^{-1}
60	7.50×10^{-1}	2.70×10^{-1}
80	4.20×10^{-1}	1.33×10^{-2}
100	3.20×10^{-1}	3.10×10^{-3}
120	1.60×10^{-1}	5.00×10^{-4}
Replicate experiment		
0	1.0	1.0
20	9.90×10^{-1}	9.50×10^{-1}
40	9.70×10^{-1}	8.70×10^{-1}
60	8.90×10^{-1}	1.89×10^{-1}
80	5.70×10^{-1}	7.90×10^{-3}
100	3.40×10^{-1}	1.99×10^{-3}
120	1.32×10^{-1}	1.04×10^{-3}

TABLE 16

Date pertaining to fig. 37 A and B

FIGURE 37A (B/r)

TIME OF IRRADIATION (min)	SURVIVING FRACTION 24 h CULTURE	4 h CULTURE	2 h CULTURE
0	1.00	1.00	1.00
20	8.50×10^{-1}	8.70×10^{-1}	8.30×10^{-1}
40	8.60×10^{-1}	4.80×10^{-1}	1.13×10^{-1}
60	8.00×10^{-1}	2.20×10^{-1}	7.00×10^{-4}
80	7.30×10^{-1}	1.40×10^{-1}	3.60×10^{-4}
100	1.43×10^{-1}	9.50×10^{-2}	1.80×10^{-4}
120	8.90×10^{-2}	4.20×10^{-3}	-

Replicate experiment

0	1.00	1.00	1.00
20	7.80×10^{-1}	8.9×10^{-1}	4.20×10^{-1}
40	6.70×10^{-1}	4.5×10^{-1}	2.00×10^{-2}
60	6.90×10^{-1}	1.35×10^{-1}	3.10×10^{-3}
80	3.90×10^{-1}	1.18×10^{-1}	7.00×10^{-4}
100	1.60×10^{-1}	1.00×10^{-1}	6.00×10^{-5}
120	6.90×10^{-2}	2.1×10^{-2}	2.00×10^{-5}

FIGURE 37B (AB1157)

0	1.00	1.00	1.00
20	7.90×10^{-1}	-	9.60×10^{-1}
40	2.47×10^{-1}	8.10×10^{-1}	8.70×10^{-1}
60	2.90×10^{-2}	2.80×10^{-1}	4.20×10^{-1}
80	5.40×10^{-3}	8.90×10^{-2}	1.76×10^{-1}
100	1.47×10^{-3}	3.75×10^{-2}	8.43×10^{-2}
120	8.30×10^{-4}	1.03×10^{-2}	7.25×10^{-2}

Replicate experiment

0	1.00	1.00	1.00
20	6.00×10^{-1}	7.90×10^{-1}	9.50×10^{-1}
40	3.60×10^{-1}	7.70×10^{-1}	5.00×10^{-1}
60	3.27×10^{-2}	3.30×10^{-1}	2.90×10^{-1}
80	3.00×10^{-3}	1.25×10^{-1}	2.48×10^{-1}
100	2.30×10^{-3}	5.20×10^{-2}	1.72×10^{-1}
120	1.70×10^{-3}	-	5.20×10^{-2}

TABLE 17

Data pertaining to fig. 38A and B

FIGURE 38A: *E. coli* B/r

GROWTH IN DEFINED MEDIA (hours)	OPTICAL DENSITY OF CULTURE AT 470 nm	SURVIVING FRACTION AFTER 100 min NUV IRRADIATION
0	0.024	1.36
1.0	-	1.50×10^{-1}
2.0	0.024	1.30×10^{-3}
2.5	0.029	1.60×10^{-2}
3.0	0.037	1.09×10^{-1}
3.5	0.060	1.68×10^{-1}
4.0	0.091	4.90×10^{-2}
4.5	0.129	2.36×10^{-1}
4.75	0.174	3.80×10^{-1}
6.0	0.460	2.80×10^{-1}
7.0	0.840	8.40×10^{-1}

Figure 38B: *E. coli* KR AB1157

0	0.018	2.00×10^{-3}
0.5	0.020	-
1.0	-	5.80×10^{-1}
1.5	0.026	-
2.0	0.041	2.50×10^{-1}
2.5	0.056	-
3.0	0.086	7.30×10^{-2}
3.5	0.120	-
4.0	0.151	2.50×10^{-2}
4.5	0.211	-
5.0	0.282	1.50×10^{-3}
6.0	0.455	3.20×10^{-3}
7.0	0.780	1.60×10^{-3}
8.0	1.130	5.10×10^{-3}

TABLE 18

Data pertaining to fig. 39

GROWTH IN DEFINED MEDIA (hours)	OPTICAL DENSITY OF CULTURE AT 470 nm	SURVIVING FRACTION AFTER 100 min NUV IRRADIATION
0	0.075	1.24
1	0.129	9.60×10^{-3}
2	0.171	-
3	0.318	7.00×10^{-3}
4	0.720	1.02
5	1.140	-
6	1.440	8.20×10^{-1}
7	1.740	8.90×10^{-1}
8	2.100	7.60×10^{-1}
9	2.300	6.70×10^{-1}
10	2.350	4.90×10^{-1}
11	-	-
12	1.725	1.08
13	1.800	4.40×10^{-1}
14	1.695	8.00×10^{-1}
15	1.680	8.30×10^{-1}
16	1.740	1.98
17	1.770	-
18	1.860	1.00
19	1.905	1.11
20	1.920	7.30×10^{-1}
21	1.800	7.60×10^{-1}
22	1.850	5.20×10^{-1}

TABLE 19

Data pertaining to fig. 40

GROWTH IN DEFINED MEDIA (hours)	OPTICAL DENSITY OF CULTURE AT 470 nm	SURVIVING FRACTION AFTER 100 min NUV IRRADIATION
0	0.015	1.79×10^{-3}
1	0.015	8.26×10^{-2}
2	0.025	2.87×10^{-1}
3	0.038	8.29×10^{-2}
4	0.081	4.10×10^{-2}
5	0.100	4.40×10^{-2}
6	0.243	1.32×10^{-2}
7	0.558	-
8	0.669	1.25×10^{-3}
9	0.705	-
10	0.795	2.40×10^{-3}
11	-	-
12	0.810	3.20×10^{-3}
13	0.815	2.70×10^{-3}
14	0.805	1.67×10^{-3}
16	0.825	8.70×10^{-4}
18	0.819	8.50×10^{-4}
20	0.858	1.79×10^{-3}
22	0.829	7.40×10^{-4}

TABLE 20

Data pertaining to fig. 41

GROWTH IN DEFINED MEDIA (hours)	OPTICAL DENSITY AT 470 nm USING 1 ml INOCULUM	USING 0.1 ml INOCULUM
0	0.013	0.001
1.0	0.024	0.001
2.0	0.075	0.004
2.5	0.125	0.009
3.0	0.190	0.020
4.0	0.380	0.065
5.0	0.524	0.188
5.5	0.590	0.300
6.0	0.650	0.450
6.5	0.750	0.670
7.0	0.810	0.810
8.0	0.910	0.990
24.0	1.830	2.150

TABLE 21

Data pertaining to fig. 42

GROWTH IN DEFINED MEDIA (hours)	VIABLE COUNT (CFU/ml)	SURVIVING FRACTION AFTER 60 min NUV IRRADIATION
0	1.60×10^{4}	1.01
0.5	1.65×10^{4}	9.00×10^{-1}
1.0	1.49×10^{4}	6.80×10^{-1}
1.5	2.14×10^{4}	5.00×10^{-2}
2.0	4.70×10^{4}	2.00×10^{-2}
2.5	7.00×10^{4}	4.00×10^{-1}
3.0	1.16×10^{5}	4.90×10^{-1}
3.5	1.90×10^{5}	4.30×10^{-1}
4.0	2.30×10^{5}	6.60×10^{-1}
4.5	7.20×10^{5}	5.00×10^{-1}
5.0	9.90×10^{5}	7.80×10^{-1}
6.0	2.58×10^{6}	5.60×10^{-1}
7.0	6.45×10^{6}	6.50×10^{-1}
Replicate experiment		
0	1.55×10^{4}	7.00×10^{-1}
0.5	1.60×10^{4}	8.40×10^{-1}
1.0	1.62×10^{4}	4.30×10^{-1}
1.5	1.88×10^{4}	9.60×10^{-2}
2.0	3.80×10^{4}	1.15×10^{-1}
2.5	7.90×10^{4}	3.80×10^{-1}
3.0	1.45×10^{5}	4.30×10^{-1}
4.0	3.20×10^{5}	5.20×10^{-1}
5.0	9.00×10^{5}	6.90×10^{-1}
6.0	2.20×10^{6}	3.40×10^{-1}
7.0	5.25×10^{6}	6.20×10^{-1}

TABLE 22

Data pertaining to fig. 43

GROWTH IN DEFINED MEDIA (hours)	VIABLE COUNT (CFU/ml)	SURVIVING FRACTION AFTER 60 min NUV IRRADIATION
0	2.81×10^{5}	3.70×10^{-1}
0.5	4.67×10^{5}	4.10×10^{-1}
1.0	1.14×10^{6}	1.03
1.5	2.37×10^{6}	6.80×10^{-1}
2.0	3.05×10^{6}	8.70×10^{-1}
2.5	5.50×10^{6}	7.00×10^{-1}
3.0	7.10×10^{6}	6.50×10^{-1}
3.5	1.20×10^{7}	6.20×10^{-1}
4.0	2.45×10^{7}	8.40×10^{-1}
Replicate experiment		
0	4.10×10^{5}	5.8×10^{-1}
1	1.10×10^{6}	7.9×10^{-1}
2	2.11×10^{6}	5.8×10^{-1}
3	6.30×10^{6}	7.1×10^{-1}
4	1.19×10^{7}	7.7×10^{-1}

TABLE 23

Data pertaining to fig. 44

GROWTH IN DEFINED MEDIA (hours)	VIABLE COUNT (CFU/ml)	SURVIVING FRACTION AFTER 60 min NUV IRRADIATION
0	6.70×10^{4}	6.00×10^{-4}
0.5	8.90×10^{4}	-
1.0	1.54×10^{5}	4.40×10^{-2}
1.5	2.49×10^{5}	-
2.0	4.50×10^{5}	1.62×10^{-1}
2.5	8.40×10^{5}	-
3.0	1.33×10^{6}	7.70×10^{-2}
3.5	2.05×10^{6}	-
4.0	2.73×10^{6}	8.86×10^{-2}
4.5	4.80×10^{6}	-
5.0	8.30×10^{6}	8.40×10^{-2}
5.5	1.16×10^{7}	-
6.0	1.88×10^{7}	1.12×10^{-2}
7.0	4.10×10^{7}	9.30×10^{-3}

Replicate experiment

0	2.44×10^{4}	4.00×10^{-4}
0.5	4.00×10^{4}	-
1.0	6.90×10^{4}	2.60×10^{-2}
1.5	8.70×10^{4}	-
2.0	1.44×10^{5}	1.35×10^{-1}
2.5	4.20×10^{5}	-
3.0	5.40×10^{5}	1.04×10^{-1}
3.5	8.60×10^{5}	-
4.0	1.43×10^{6}	3.00×10^{-2}
4.5	3.50×10^{6}	-
5.0	4.30×10^{6}	5.50×10^{-2}
5.5	9.90×10^{6}	-
6.0	1.38×10^{7}	8.50×10^{-3}
6.5	4.90×10^{7}	-
7.0	4.20×10^{7}	4.00×10^{-3}

TABLE 24

Data pertaining to fig. 45A and B

FIGURE 45A: *E. coli* SR 834

GROWTH IN DEFINED MEDIA (hours)	OPTICAL DENSITY AT 470 nm CONTROL SAMPLE	IRRADIATED SAMPLE
0	0.050	0.040
0.25	0.043	0.028
0.50	0.055	0.051
0.75	0.089	0.034
1.00	0.110	0.039
1.25	0.131	0.050
1.50	0.155	0.054
1.75	0.205	0.070
2.00	0.260	0.100
2.25	0.322	0.104
2.50	0.400	0.113
2.75	0.471	0.152
3.00	0.565	0.191
3.25	0.653	0.256
3.50	-	0.298
3.75	-	0.362
4.00	-	0.438
4.25	-	0.535

Replicate experiment

0	0.043	0.023
0.25	0.028	0.035
0.50	0.045	0.015
0.75	0.055	0.025
1.00	0.049	0.040
1.25	0.056	0.023
1.50	0.079	0.034
1.75	0.091	0.046
2.00	0.125	0.046
2.25	0.144	0.049
2.50	0.194	0.056
2.75	0.240	0.075
3.00	0.290	0.090
3.25	0.344	0.145
3.50	-	0.140
3.75	-	0.186
4.00	-	0.215
4.25	-	0.270

FIGURE 45B: *E. coli* SR 833

0	0.040	0.032
0.50	0.049	0.032
1.00	0.084	0.030
1.50	0.135	0.037
2.00	0.190	0.046
2.50	0.320	0.034
3.00	0.435	0.045
3.50	0.595	0.037
4.00	0.740	0.044
4.50	0.850	0.054
5.00	-	0.063
5.25	-	0.069
5.50	-	0.087
5.75	-	0.094
6.00	-	0.110
6.25	-	0.124
6.50	-	0.156
6.75	-	0.204
7.00	-	0.220

Replicate experiment

0	0.028	0.036
0.25	0.060	0.037
0.50	0.061	0.039
0.75	0.066	0.046
1.00	0.085	0.040
1.25	0.117	0.038
1.50	0.127	0.060
1.75	0.140	0.040
2.00	0.171	0.050
2.25	0.204	0.052
2.50	0.253	0.040
2.75	0.295	0.053
3.00	0.335	0.041
3.25	0.382	0.046
3.50	-	0.064
3.75	-	0.052
4.00	-	0.065
4.25	-	0.066

TABLE 25

Data pertaining to fig. 46

TIME OF IRRADIATION (min)	SURVIVING FRACTION *E. coli* SR833	*E. coli* SR834
0	1.00	1.00
10	-	1.06
20	7.00×10^{-1}	1.01
40	8.30×10^{-2}	6.90×10^{-1}
60	9.60×10^{-3}	3.80×10^{-1}
80	3.70×10^{-4}	1.50×10^{-1}
100	1.50×10^{-4}	1.70×10^{-2}
120	2.00×10^{-5}	6.00×10^{-4}

Replicate experiment

0	1.00	1.00
10	1.67×10^{-1}	9.60×10^{-1}
20	6.90×10^{-2}	1.19
40	3.90×10^{-3}	8.40×10^{-1}
60	1.90×10^{-3}	6.70×10^{-1}
80	1.30×10^{-4}	2.00×10^{-1}
100	6.00×10^{-5}	3.70×10^{-2}
120	4.00×10^{-5}	2.20×10^{-3}

TABLE 26

Data pertaining to fig. 47

GROWTH IN DEFINED MEDIA (hours)	OPTICAL DENSITY AT 470 nm		SURVIVING FRACTION AFTER 60 min NUV IRRADIATION	
	SR833	SR834	SR833	SR834
0	0.033	0.031	6.40×10^{-1}	1.00
1	0.034	0.036	4.30×10^{-1}	9.10×10^{-1}
2	0.060	0.044	4.00×10^{-1}	5.30×10^{-1}
3	0.105	0.067	1.70×10^{-2}	3.20×10^{-1}
4	0.240	0.185	2.30×10^{-1}	9.60×10^{-1}
5	0.410	0.383	2.90×10^{-1}	5.90×10^{-1}
6	0.550	0.580	7.50×10^{-1}	8.90×10^{-1}
7	0.650	0.720	8.40×10^{-1}	7.90×10^{-1}
7.75	0.740	0.830	6.70×10^{-1}	8.10×10^{-1}
Replicate experiment				
0	0.038	0.040	8.90×10^{-1}	1.05
1	0.039	0.042	4.70×10^{-1}	9.90×10^{-1}
2	0.055	0.056	3.20×10^{-1}	8.30×10^{-1}
3	0.092	0.096	1.08×10^{-2}	7.80×10^{-1}
4	0.190	0.195	6.70×10^{-2}	8.50×10^{-1}
5	0.462	0.453	7.10×10^{-1}	5.70×10^{-1}
6	0.521	0.499	5.10×10^{-1}	9.90×10^{-1}
7	0.660	0.651	7.80×10^{-1}	8.20×10^{-1}

TABLE 27

Data pertaining to fig. 48A and B

FIGURE 48A: *E. coli* NF161

TIME OF IRRADIATION (min)	SURVIVING FRACTION 3h	6h	24h
0	1.00	1.00	1.00
20	6.60×10^{-1}	1.06	-
40	8.10×10^{-2}	1.04	8.80×10^{-1}
60	3.20×10^{-2}	9.40×10^{-1}	8.10×10^{-1}
80	6.80×10^{-3}	5.70×10^{-1}	8.80×10^{-1}
100	2.11×10^{-3}	1.20×10^{-1}	6.20×10^{-1}
120	8.00×10^{-4}	-	7.20×10^{-1}

Replicate experiment

0	1.00	1.00	1.00
20	6.30×10^{-1}	9.60×10^{-1}	9.10×10^{-1}
40	7.90×10^{-2}	9.70×10^{-1}	9.60×10^{-1}
60	2.30×10^{-2}	9.20×10^{-1}	8.20×10^{-1}
80	7.40×10^{-3}	5.50×10^{-1}	8.50×10^{-1}
100	1.90×10^{-3}	1.20×10^{-1}	8.20×10^{-1}
120	7.00×10^{-4}	8.20×10^{-3}	7.40×10^{-1}

FIGURE 48B: *E. coli* NF162

0	1.00	1.00	1.00
20	1.06	4.60×10^{-1}	-
40	6.50×10^{-1}	1.50×10^{-1}	8.70×10^{-1}
60	6.90×10^{-1}	7.20×10^{-2}	4.10×10^{-1}
80	5.00×10^{-1}	1.20×10^{-2}	1.70×10^{-1}
100	9.70×10^{-2}	1.40×10^{-3}	1.13×10^{-1}
120	7.90×10^{-2}	-	4.20×10^{-2}

Replicate experiment

0	1.00	1.00	1.00
20	9.80×10^{-1}	4.40×10^{-1}	1.00
40	5.60×10^{-1}	1.15×10^{-1}	9.50×10^{-1}
60	4.90×10^{-1}	6.50×10^{-2}	5.90×10^{-1}
80	3.50×10^{-1}	1.35×10^{-2}	1.62×10^{-1}
100	8.70×10^{-2}	1.70×10^{-3}	9.54×10^{-2}
120	6.20×10^{-2}	2.60×10^{-4}	2.92×10^{-2}

TABLE 28

Data pertaining to fig. 49

GROWTH IN DEFINED MEDIA (hours)	OPTICAL DENSITY AT 470 nm		SURVIVING FRACTION AFTER 90 min NUV IRRADIATION	
	NF161	NF162	NF161	NF162
0	0.030	0.032	8.20×10^{-1}	5.40×10^{-1}
1	0.035	0.036	5.10×10^{-1}	4.90×10^{-1}
2	0.049	0.055	2.07×10^{-1}	6.30×10^{-1}
3	0.118	0.090	3.50×10^{-2}	6.30×10^{-1}
4	0.275	0.186	2.90×10^{-3}	2.90×10^{-1}
5	0.555	0.379	1.30×10^{-3}	2.40×10^{-1}
6	0.780	0.725	7.80×10^{-2}	8.20×10^{-3}
7	0.940	0.910	4.70×10^{-1}	2.70×10^{-2}
8	1.080	1.080	6.80×10^{-1}	3.75×10^{-2}
9	1.180	1.180	9.40×10^{-1}	3.60×10^{-2}
10	1.270	1.210	8.00×10^{-1}	9.10×10^{-2}

TABLE 29

Data pertaining to fig. 50

TIME OF IRRADIATION (min)	SURVIVING FRACTION		
	YENB	TA	DEFINED
0	1.00	1.00	1.00
5	1.15	1.05	1.05
10	1.11	1.13	8.80×10^{-1}
20	1.13	1.05	7.30×10^{-1}
40	1.06	1.08	3.70×10^{-1}
60	2.70×10^{-1}	5.10×10^{-1}	1.80×10^{-1}
80	5.10×10^{-2}	1.86×10^{-1}	9.30×10^{-2}
100	7.20×10^{-3}	1.86×10^{-2}	2.20×10^{-2}
120	9.00×10^{-4}	2.00×10^{-3}	6.70×10^{-3}
Replicate experiment			
0	1.00	1.00	1.00
10	1.08	1.11	9.20×10^{-1}
20	1.05	1.09	5.90×10^{-1}
40	1.01	1.08	3.30×10^{-1}
60	1.90×10^{-1}	4.10×10^{-1}	1.15×10^{-1}
80	6.00×10^{-2}	6.20×10^{-2}	1.00×10^{-1}
100	4.50×10^{-3}	2.10×10^{-2}	3.60×10^{-2}

TABLE 30

Data pertaining to fig. 51

TIME OF IRRADIATION (min)	SURVIVING FRACTION YENB	TA	DEFINED	SR 385
0	1.00	1.00	1.00	1.00
5	6.60×10^{-1}	9.60×10^{-1}	2.35×10^{-1}	-
10	7.30×10^{-2}	6.20×10^{-1}	1.17×10^{-1}	-
15	9.30×10^{-3}	-	-	-
20	3.00×10^{-3}	2.80×10^{-1}	1.40×10^{-2}	9.50×10^{-1}
25	1.60×10^{-3}	-	-	-
30	9.00×10^{-4}	-	-	-
40	-	2.10×10^{-3}	-	4.40×10^{-1}
60	-	-	-	3.50×10^{-1}
80	-	-	-	8.50×10^{-2}

Replicate experiment

0	1.00	1.00	1.00	1.00
5	8.00×10^{-1}	-	2.46×10^{-1}	-
10	8.90×10^{-2}	5.90×10^{-1}	7.56×10^{-2}	1.01
15	1.90×10^{-3}	-	1.44×10^{-2}	-
20	1.40×10^{-4}	2.10×10^{-1}	5.00×10^{-3}	9.90×10^{-1}
30	1.40×10^{-5}	4.10×10^{-2}	2.00×10^{-4}	-
40	-	-	-	5.25×10^{-1}
60	-	-	-	-
80	-	-	-	7.60×10^{-2}

TABLE 31

Data pertaining to figs. 52 and 53

FIGURE 52

TIME OF IRRADIATION (min)	SURVIVING FRACTION NB	DEFINED
0	1.00	1.00
5	2.80×10^{-1}	8.20×10^{-1}
10	2.50×10^{-1}	6.60×10^{-1}
15	1.40×10^{-1}	6.70×10^{-1}
20	2.10×10^{-2}	5.70×10^{-1}
30	5.00×10^{-4}	3.60×10^{-1}
40	-	1.30×10^{-2}

FIGURE 53

FLUENCE (Jm^{-2})	SURVIVING FRACTION YENB	DEFINED
0	1.00	1.00
35.4	3.60×10^{-1}	5.00×10^{-1}
70.8	1.07×10^{-1}	1.90×10^{-1}
106.2	3.60×10^{-2}	7.00×10^{-2}
141.6	6.80×10^{-3}	2.40×10^{-2}
177.0	8.00×10^{-4}	4.20×10^{-3}

Replicate experiment

0	1.00	1.00
35.4	3.50×10^{-1}	4.90×10^{-1}
70.8	7.20×10^{-2}	1.74×10^{-1}
106.2	1.70×10^{-2}	8.90×10^{-2}
141.6	3.40×10^{-3}	3.00×10^{-2}
177.0	6.00×10^{-4}	5.30×10^{-3}

TABLE 32

Data pertaining to figs. 54 and 55

FIGURE 54

FLUENCE ($Jm^{-2}x10^{5}$)	SURVIVING FRACTION		
	SR246	BW6163	X14/501
0	1.00	1.00	1.00
0.88	$9.40x10^{-1}$	$8.70x10^{-1}$	1.26
1.76	$6.50x10^{-1}$	$7.50x10^{-1}$	1.20
2.64	$2.90x10^{-2}$	$5.50x10^{-1}$	$9.70x10^{-1}$
3.53	$2.70x10^{-4}$	$5.40x10^{-1}$	$9.40x10^{-1}$
4.41	$2.00x10^{-5}$	$3.20x10^{-1}$	1.13
5.30	-	$1.40x10^{-1}$	$8.80x10^{-1}$

FIGURE 55

FLUENCE ($Jm^{-2}x10^{5}$)	SURVIVING FRACTION	
	NB	DEFINED
0	1.00	1.00
0.56	1.09	$8.90x10^{-1}$
1.11	$5.20x10^{-1}$	1.00
1.67	$2.90x10^{-1}$	$7.30x10^{-1}$
2.23	$1.21x10^{-1}$	$4.00x10^{-1}$
2.78	$9.40x10^{-3}$	$9.50x10^{-2}$
3.34	$3.20x10^{-4}$	$5.50x10^{-2}$
3.90	$9.00x10^{-5}$	$1.60x10^{-2}$
4.45	-	$8.40x10^{-3}$
5.01	-	$5.50x10^{-4}$

TABLE 33

Data pertaining to fig. 56

TIME OF IRRADIATION (mins)	SURVIVING FRACTION YENB	YENB+ CATALASE	YENB+ INACTIVATED CATALASE
0	1.00	1.00	1.00
5	5.90×10^{-1}	6.60×10^{-1}	4.50×10^{-1}
10	1.07×10^{-1}	5.10×10^{-1}	1.50×10^{-2}
15	1.53×10^{-2}	5.20×10^{-1}	6.60×10^{-3}
20	7.60×10^{-4}	2.70×10^{-1}	4.50×10^{-4}
25	1.80×10^{-4}	1.48×10^{-1}	5.00×10^{-5}
30	8.00×10^{-5}	9.00×10^{-2}	3.00×10^{-5}

Replicate experiment

0	1.00	1.00
5	8.00×10^{-1}	6.20×10^{-1}
10	8.90×10^{-2}	6.50×10^{-1}
15	1.90×10^{-3}	6.30×10^{-1}
20	1.40×10^{-4}	7.80×10^{-1}
30	1.40×10^{-5}	5.10×10^{-1}

SR385	SURVIVING FRACTION ASSESSED ON YENB		
	EXPT. 1	EXPT. 2	MEAN
0	1.00	1.00	1.00
10	9.60×10^{-1}	9.20×10^{-1}	9.40×10^{-1}
20	9.20×10^{-1}	8.70×10^{-1}	8.85×10^{-1}
30	5.40×10^{-1}	6.50×10^{-1}	5.85×10^{-1}

TABLE 34

Data pertaining to fig. 57

TIME OF EXPOSURE TO 5 mM H_2O_2 (mins)	CHANGE IN OPTICAL DENSITY AT 405 nm FOR STATIONARY PHASE CELLS		
	SR246	UMI	SR385
0	0	0	0
2.5	0.019	0.005	-
5.0	0.061	0	0.084
7.5	0.082	0.003	-
10.0	0.110	0.010	0.139
12.5	0.135	0.007	-
15.0	0.150	0.016	0.165
20.0	0.177	0.019	0.204
25.0	0.193	0.017	-
30.0	0.199	0.019	0.235

Replicate experiment

0	0	0	0
2.5	0.027	0.012	-
5.0	0.086	0.006	0.072
7.5	0.102	0.010	-
10.0	0.148	0.011	0.144
15.0	0.177	0.027	0.172
20.0	0.202	0.023	0.210
25.0	0.221	0.026	-
30.0	0.238	0.027	0.224

TABLE 35

Data pertaining to fig. 58

TIME OF IRRADIATION (mins)	SURVIVING FRACTION STATIONARY PHASE CELLS	EXPONENTIAL PHASE CELLS
0	1.00	1.00
5	8.30×10^{-1}	9.80×10^{-1}
10	7.80×10^{-3}	7.40×10^{-1}
15	6.00×10^{-4}	1.10
20	2.60×10^{-4}	7.50×10^{-1}
25	1.60×10^{-4}	6.20×10^{-1}
30	-	5.50×10^{-1}
45	-	1.40×10^{-2}

Replicate experiment

0	1.00	1.00
5	8.20×10^{-1}	8.90×10^{-1}
10	1.33×10^{-2}	8.90×10^{-1}
15	1.52×10^{-3}	8.20×10^{-1}
20	3.80×10^{-4}	7.30×10^{-1}
25	2.80×10^{-4}	6.30×10^{-1}
30	4.70×10^{-5}	5.40×10^{-1}
45	-	3.02×10^{-2}

TABLE 36

Data pertaining to fig. 60

LENGTH OF CONJUGATION (min)	NUMBER OF RECOMBINANTS PER ml GROWN ON his^-,str^+	his^-,str^+,tet^+
0	$1.00x10^2$	0
2	0	0
4	0	$2.00x10^2$
6	0	0
8	$3.00x10^2$	$1.00x10^2$
10	0	0
12	$4.00x10^2$	$1.00x10^2$
14	$1.30x10^3$	0
16	$3.20x10^3$	0
18	$4.70x10^3$	$1.00x10^2$
20	$1.10x10^4$	$1.00x10^2$
22	$1.53x10^4$	$8.00x10^2$
24	$1.90x10^4$	$1.10x10^3$
26	$2.10x10^4$	$1.70x10^3$
28	$2.64x10^4$	$2.70x10^3$
30	$3.02x10^4$	$5.50x10^3$
35	$4.39x10^4$	$7.30x10^3$
40	$4.30x10^4$	$1.55x10^4$

(0 - no visible colonies)

TABLE 37

Data pertaining to fig. 61

LENGTH OF CONJUGATION (min)	NUMBER OF RECOMBINANTS PER ml GROWN ON	
	his^-,str^+	his^-,str^+,tet^+
0	0	0
10	2.00×10^2	0
14	3.00×10^2	0
18	6.00×10^2	0
20	4.00×10^2	0
22	5.00×10^2	1.00×10^2
24	1.70×10^3	3.00×10^2
26	1.03×10^4	2.00×10^3
28	2.09×10^4	4.50×10^3
30	2.78×10^4	-
35	3.50×10^4	1.37×10^4
40	6.00×10^4	2.10×10^4

(0 - no visible colonies)

TABLE 38

Data pertaining to fig. 62

REPLICATE NUMBER	SURVIVING FRACTION AFTER NEAR-UV IRRADIATION		
	0 min	15 min	30 min
1	1.00	2.90×10^{-2}	1.40×10^{-4}
2	1.00	1.80×10^{-2}	5.00×10^{-5}
3	1.00	1.80×10^{-2}	3.00×10^{-5}
4	1.00	2.30×10^{-2}	3.00×10^{-5}
5	1.00	3.15×10^{-2}	3.00×10^{-5}
6	1.00	3.80×10^{-2}	6.00×10^{-5}
7	1.00	2.80×10^{-2}	5.00×10^{-5}
8	1.00	1.30×10^{-2}	6.00×10^{-6}
9	1.00	6.60×10^{-3}	3.00×10^{-6}

TABLE 39

Data pertaining to fig. 63

Hfr-Tn10 STRAIN CODE	SURVIVING FRACTION AFTER 30 min NEAR-UV IRRADIATION EXPT. 1	EXPT. 2	MEAN
1	6.00×10^{-3}	5.20×10^{-2}	2.90×10^{-2}
2	6.10×10^{-1}	7.50×10^{-1}	6.80×10^{-1}
3	4.00×10^{-3}	6.40×10^{-1}	3.22×10^{-1}
4	1.20×10^{-1}	7.20×10^{-1}	4.20×10^{-1}
5	2.10×10^{-1}	6.00×10^{-1}	4.05×10^{-1}
6	5.80×10^{-1}	1.41	9.95×10^{-1}
7	1.38×10^{-2}	1.06×10^{-1}	5.99×10^{-2}
8	1.50×10^{-3}	1.80×10^{-1}	9.07×10^{-2}
9	9.60×10^{-1}	6.06×10^{-1}	7.83×10^{-1}
10	1.07	9.80×10^{-1}	1.03
11	8.00×10^{-2}	1.16×10^{-1}	9.80×10^{-2}
12	9.50×10^{-1}	8.60×10^{-1}	9.05×10^{-1}
13	1.20×10^{-1}	4.70×10^{-1}	2.95×10^{-1}
14	5.30×10^{-1}	1.21	8.70×10^{-1}
15	4.70×10^{-1}	8.90×10^{-1}	6.80×10^{-1}
16	1.05×10^{-2}	1.30×10^{-3}	5.90×10^{-3}
SR246	6.00×10^{-4}	1.00×10^{-4}	3.50×10^{-4}
	4.00×10^{-5}	1.70×10^{-5}	2.85×10^{-5}
	6.00×10^{-5}	3.00×10^{-5}	4.50×10^{-5}

TABLE 40

Data pertaining to fig. 64

SR246 x Hfr-Tn10 CONJUGANT CODE	SURVIVING FRACTION AFTER 30 min NEAR-UV IRRADIATION
X3	6.00×10^{-6}
X4	2.50×10^{-4}
X5	1.09×10^{-3}
X6	3.30×10^{-4}
X9	4.70×10^{-1}
X10	5.00×10^{-5}
X13	1.34×10^{-3}
X14	5.20×10^{-2}
X15	4.30×10^{-1}
SR246	6.00×10^{-5}
SR246	5.00×10^{-5}
SR246	4.40×10^{-4}

TABLE 41

Data pertaining to fig. 65

STRAIN	SURVIVING FRACTION AFTER 30 min NEAR-UV IRRADIATION
X9 CONJUGANT	
REPLICATE 1	2.20×10^{-1}
REPLICATE 2	1.60×10^{-1}
REPLICATE 3	2.70×10^{-1}
REPLICATE 4	3.20×10^{-1}
REPLICATE 5	2.10×10^{-1}
REPLICATE 6	4.10×10^{-1}
REPLICATE 7	2.30×10^{-1}
REPLICATE 8	2.90×10^{-1}
REPLICATE 9	1.50×10^{-1}
BW6156	
REPLICATE 1	8.50×10^{-1}
REPLICATE 2	8.60×10^{-1}
REPLICATE 3	1.04
SR246	
REPLICATE 1	2.70×10^{-5}
REPLICATE 2	9.00×10^{-5}
REPLICATE 3	2.00×10^{-5}

TABLE 42

Data pertaining to figs. 66 and 67

FIGURE 66

LENGTH OF CONJUGATION (min)	VIABLE COUNT CFU/ml REPLICATE 1	REPLICATE 2	MEAN
0	1.00×10^{2}	3.00×10^{2}	2.00×10^{2}
10	2.00×10^{2}	2.00×10^{2}	2.00×10^{2}
15	7.00×10^{1}	1.30×10^{2}	1.00×10^{2}
20	5.00×10^{2}	7.00×10^{2}	6.00×10^{2}
25	3.50×10^{3}	3.10×10^{3}	3.30×10^{3}
30	1.35×10^{4}	1.85×10^{4}	1.60×10^{4}
35	1.86×10^{4}	2.38×10^{4}	2.12×10^{4}
40	4.32×10^{4}	4.64×10^{4}	4.48×10^{4}
45	4.88×10^{4}	5.52×10^{4}	5.20×10^{4}
50	7.02×10^{4}	8.66×10^{4}	7.84×10^{4}

FIGURE 67

CONJUGANT OR STRAIN	SURVIVING FRACTION AFTER 30 min NEAR-UV IRRADIATION REPLICATE 1	REPLICATE 2	REPLICATE 3
X9/25	1.70×10^{-5}	7.50×10^{-5}	4.00×10^{-6}
X9/30	2.50×10^{-6}	-	-
X9/35	5.00×10^{-5}	4.00×10^{-6}	1.30×10^{-5}
X9/40	1.60×10^{-4}	7.00×10^{-6}	2.00×10^{-6}
X9/50	1.00×10^{-5}	7.00×10^{-5}	3.00×10^{-5}
BW6156	8.40×10^{-1}	9.70×10^{-1}	7.90×10^{-1}
SR246	2.40×10^{-5}	3.60×10^{-5}	2.40×10^{-5}

TABLE 43

Data pertaining to fig. 69

	STRAIN OR CONJUGANT	SURVIVING FRACTION AFTER 30 min NEAR-UV IRRADIATION (REPLICATES)
(A)	SR246	5.00×10^{-5}, 1.30×10^{-4}, 1.60×10^{-4}
	BW6169	1.1, 1.03, 1.06
	X15/30	4.70×10^{-4}, 1.80×10^{-3}, 1.60×10^{-4}
	X15/40	1.70×10^{-3}, 5.40×10^{-3}, 3.00×10^{-5}
		2.00×10^{-5}, 5.00×10^{-5}, 1.00×10^{-5}
	X15/50	3.00×10^{-5}, 5.00×10^{-5}, 3.10×10^{-1}
		1.40×10^{-4}, 3.00×10^{-5}, 7.00×10^{-5}
		1.20×10^{-3}, 7.00×10^{-5}, 1.80×10^{-4}
(B)	SR246	1.60×10^{-3}, 1.00×10^{-5}, -
	BW6163	1.32, 9.90×10^{-1}, -
	X14/30	6.80×10^{-2}, 1.03×10^{-2}, 3.90×10^{-3}
		7.00×10^{-6}, 9.70×10^{-2}, 2.02×10^{-1}
	X14.50	3.40×10^{-4}, 1.22×10^{-1}, 2.70×10^{-2}
		1.09×10^{-1}, 1.13×10^{-1}, 6.60×10^{-2}
(C)	SR246	1.00×10^{-3}, 2.00×10^{-4}, -
	BW5659	5.56×10^{-1}, 7.60×10^{-1}, -
	X13/26	1.67×10^{-2}, 5.00×10^{-5}, 3.80×10^{-2}
		1.15×10^{-2}, 3.00×10^{-4}, 1.20×10^{-3}
	X13/50	1.40×10^{-4}, 5.60×10^{-3}, 5.60×10^{-4}
		1.05×10^{-3}, 2.40×10^{-3}, 2.00×10^{-5}

TABLE 44

Data pertaining to fig. 70

	STRAIN OR CONJUGANT	SURVIVING FRACTION AFTER 30 min NEAR-UV IRRADIATION (REPLICATES)		
(A)	SR246	1.50×10^{-3}	1.09×10^{-3}	-
	BW7623	6.34×10^{-1}	8.00×10^{-1}	-
	X11/35	9.70×10^{-3}	3.10×10^{-3}	1.50×10^{-4}
		9.00×10^{-5}	3.30×10^{-3}	2.40×10^{-3}
	X11/50	1.70×10^{-4}	2.70×10^{-3}	5.10×10^{-3}
		3.40×10^{-3}	4.80×10^{-3}	4.40×10^{-3}
(B)	SR246	2.00×10^{-4}	1.20×10^{-4}	-
	BW5660	4.40×10^{-1}	3.80×10^{-1}	-
	X3/30	3.40×10^{-3}	1.80×10^{-4}	4.80×10^{-4}
		1.80×10^{-4}	2.05×10^{-3}	2.00×10^{-5}
	X3/50	1.30×10^{-4}	1.00×10^{-4}	5.50×10^{-4}
		2.00×10^{-5}	3.00×10^{-4}	1.40×10^{-4}
(C)	SR246	5.00×10^{-6}	6.00×10^{-5}	3.00×10^{-5}
		1.00×10^{-5}	-	-
	BW6175	3.50×10^{-1}	4.40×10^{-1}	3.60×10^{-1}
		3.10×10^{-1}	-	-
	X5/20	1.40×10^{-5}	2.00×10^{-6}	6.00×10^{-6}
	X5/25	1.50×10^{-5}	4.80×10^{-5}	7.00×10^{-6}
	X5/30	4.70×10^{-4}	1.40×10^{-5}	2.70×10^{-4}
	X5/40	3.00×10^{-6}	8.00×10^{-6}	-

TABLE 45

Data pertaining to fig. 71

STRAIN OR CONJUGANT	SURVIVING FRACTION AFTER 30 min NEAR-UV IRRADIATION (REPLICATES)
SR246	3.00×10^{-5}, 2.20×10^{-3}, -
BW6163	7.30×10^{-1}, 1.22
X14/28	9.35×10^{-2}, 7.00×10^{-4}, 5.00×10^{-4}
	1.19×10^{-2}, 8.10×10^{-2}, 1.90×10^{-2}
X14/35	2.70×10^{-1}, 2.70×10^{-2}, 1.60×10^{-3}
	7.00×10^{-5}, 2.26×10^{-2}, 1.50×10^{-4}
X14/50	3.77×10^{-1}, 2.76×10^{-2}, 1.00×10^{-3}
	2.00×10^{-2}, 1.77×10^{-1}, 9.60×10^{-2}

TABLE 46

Data pertaining to fig. 72

TIME OF IRRADIATION (min)	SURVIVING FRACTION X14/50 (1)	X14/50 (2)	X14/35 (3)
0	1.00	1.00	1.00
5	4.90×10^{-1}	2.50×10^{-1}	1.29
10	2.60×10^{-1}	1.80×10^{-1}	1.07
15	3.30×10^{-1}	2.05×10^{-1}	8.50×10^{-1}
20	3.30×10^{-1}	2.50×10^{-1}	7.60×10^{-1}
25	3.08×10^{-1}	2.20×10^{-1}	4.20×10^{-1}
30	3.50×10^{-1}	1.50×10^{-1}	3.40×10^{-1}

	SR246	BW6163
0	1.00	1.00
5	6.60×10^{-1}	7.70×10^{-1}
10	7.30×10^{-2}	1.03
15	9.30×10^{-3}	8.60×10^{-1}
20	3.00×10^{-3}	1.04
25	1.60×10^{-3}	7.40×10^{-1}
30	9.00×10^{-4}	7.60×10^{-1}

REFERENCES

Adelberg E. A. and S. N. Burns (1960).
Genetic variation in the sex factor of *Escherichia coli*.
J. Bacteriol. **130**, 321-330.

Ananthaswamy H. N. and A. Eisenstark (1976).
Near-UV induced breaks in phage DNA: sensitization by H_2O_2.
Photochem. Photobiol. **24**, 439-442.

Ananthaswamy H. N. and A. Eisenstark (1977).
Repair of hydrogen peroxide-induced single-strand breaks in *Escherichia coli* deoxyribonucleic acid.
J. Bacteriol. **130**, 187-191.

Ananthaswamy H. N., P. S. Hartman and A. Eisenstark (1979).
Synergistic lethality of phage T7 by near-UV radiation and hydrogen peroxide: an action spectrum.
Photochem. Photobiol. **29**, 53-56.

Anderson E. H. (1946).
Growth requirements of virus-resistant mutants of *Escherichia coli* strain "B".
Proc. Natl. Acad. Sci. (USA). **32**, 120-128

Bezman S. A., P. A. Burtis, T. P. J. Izod, and M. A. Thayer (1978).
Photodynamic inactivation in *E. coli* by rose bengal immobilized on polystyrene beads.
Photochem. Photobiol. **28**, 325-329.

Bochner B. R., P. C. Lee, S. W. Wilson, C. W. Cutler and B. N. Ames (1984).
AppppA and related adenylylated nucleotides are synthesised as a consequence of oxidative stress.
Cell. **37**, 225-232.

Boyce R. P. and P. Howard-Flanders (1964).
Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K12.
Proc. Natl. Acad. Sci. (USA). **51**, 293-301.

Brown M. S. and R. B. Webb (1972).
Photoreactivation of 365 nm inactivation of *Escherichia coli*.
Mutat. Res. **15**, 348-352.

Cabrera-Juárez E. and J. K. Setlow (1977).
Formation of a thymine photoproduct in transforming DNA by near-UV irradiation.
Biochem. Biophys. Acta. **475**, 315-322.

Cabrera-Juárez E., J. K. Setlow, P. A. Swenson and M. J. Peak (1976).
Oxygen-independent inactivation of *Haemophilus influenzae* transforming DNA by monochromatic radiation: action spectrum, effect of histidine and repair.
Photochem. Photobiol. **23**, 309-313.

Carlsson J. and V. S. Carpenter (1980).
The $recA^+$ gene product is more important than catalase and superoxide dismutase in protecting *Escherichia coli* against hydrogen peroxide toxicity.
J. Bacteriol. **142**, 319-321.

Carré D. S., G. Thomas and A. Favre (1974).
Conformation and functioning of tRNAs: Cross-linked tRNA as substrate for tRNA nucleotidyl-transferase and aminoacyl synthetases.
Biochimie. **56**, 1089-1101.

Cashel M. (1969).
The control of ribonucleic acid synthesis in *Escherichia coli*.
J. Biol. Chem. **244**, 3133-3141.

Chamberlain J. and S. H. Moss (1987).
Lipid peroxidation and other membrane damage produced in *Escherichia coli* K1060 by near-UV radiation and deuterium oxide.
Photochem. Photobiol. **45**, 625-630.

Christman M. F., R. W. Morgan, F. S. Jacobson and B. N. Ames (1985).
Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*.
Cell. **41**, 753-762.

Claiborne A. and I. Fridovich (1979).
Purification of the o-dianisidine peroxidase from *Escherichia coli* B.
J. Biol. Chem. **254**, 4245-4252.

Daly J. J., M. L. Baker and S. B. Burton (1981).
The sensitivity of *Trichomonas vaginalis* and *Trichomonas gallinae* to ultraviolet radiation.
Photochem. Photobiol. **33**, 191-195.

Demple B. and J. Halbrook (1983).
Inducible repair of oxidative DNA damage in *Escherichia coli*.
Nature. **304**, 466-468.

Demple B. and S. Linn (1982).
5,6-saturated thymine lesions in DNA: production by ultraviolet light or hydrogen peroxide.
Nucleic Acids Res. **10**, 3781-3789.

Demple B., J. Halbrook and S. Linn (1983).
Escherichia coli xth mutants are hypersensitive to hydrogen peroxide.
J. Bacteriol. **153**, 1079-1082.

Demple B., A. Johnson and D. Fung (1986).
Exonuclease III and endonuclease IV remove 3' blocks from DNA synthesis primers in H_2O_2-damaged *Escherichia coli*.
Proc. Natl. Acad. Sci. (USA). **83**, 7731-7735.

Dulbecco R. (1949).
Reactivation of ultraviolet inactivated bacteriophage by visible light.
Nature. **163**, 949-950.

Dulbecco R. and J. J. Weigle (1952).
Inhibition of bacteriophage development in bacteria illuminated with visible light.
Experientia. **8**, 386-389.

Eisenstark A. (1970).
Sensitivity of *Salmonella typhimurium* recombinationless (*rec*) mutants to visible and near-visible light.
Mutat. Res. **10**, 1-6.

Eisenstark A. (1973).
Tryptophan photoproduct as a genetic probe: Effects on bacteria.
in: *Fifth Stadler Genetics Symp.*
ed: G. Kimber and G. P. R. Redei.
publ: Univ. Missouri. Columbia.
pp: 49-60.

Eisenstark A. and G. Perrot (1987).
Catalase has only a minor role in protection against near-ultraviolet radiation damage in bacteria.
Mol. Gen. Genet. **207**, 68-72.

Eisenstark A., R. L. Buzard and P. S. Hartman (1986).
Inactivation of phage by near-ultraviolet radiation and hydrogen peroxide.
Photochem. Photobiol. **44**, 603-606.

Farr S. B., D. O. Natvig and T. Kogoma (1985).
Toxicity and mutagenicity of plumbagin and the induction of a possible new DNA repair pathway in *Escherichia coli*.
J. Bacteriol. **164**, 1309-1316.

Farr S. B., D. Touati and T. Kogoma (1988).
Effects of oxygen stress on membrane functions in *Escherichia coli*: Role of HPI catalase.
J. Bacteriol. **170**, 1837-1842.

Favre A. and G. Thomas (1981).
Transfer RNA: from photophysics to photobiology.
Ann. Rev. Biophys. Bioeng. **10**, 175-195.

Favre A., A. M. Michelson and M. Yaniv (1971).
Photochemistry of 4-thiouridine in *Escherichia coli* transfer RNA_1^{VAL}.
J. Mol. Biol. **58**, 367-379.

Favre A., R. Buckingham and G. Thomas (1975).
Transfer-RNA tertiary structure in solution as probed by photochemically induced 8-13 cross link.
Nucl. Acid. Res. **2**, 1421-1431.

Favre A., E. Hajnsdorf, K. Thiam and A. Caldeira de Aranjo (1985).
Mutagenesis and growth delay induced in *Escherichia coli* by near-ultraviolet radiations.
Biochimie. **67**, 335-342.

Fiil N. P., B. M. Willumsen, J. D. Friesen and K. von Meyenburg (1977).
Interactions of alleles of the *relA, relC* and *spoT* genes in *Escherichia coli*: Analysis of the interconversion of GTP, ppGpp and pppGpp.
Mol. Gen. Genet. **150**, 87-101.

Finn G. J. and S. Condon (1975).
Regulation of catalase synthesis in *Salmonella typhimurium*.
J. Bacteriol. **123**, 570-579.

Fita I. and M. G. Rossman (1985).
The active centre of catalase.
J. Mol. Biol. **185**, 21-38.

Forbes P. D., R. E. Davies, L. C. D'Aloisio and C. Cole (1976).
Emission spectrum differences in fluorescent Blacklight lamps.
Photochem. Photobiol. **24**, 613-615.

Friedberg, E. C. (1985).
DNA repair.
publ: W. H. Freeman and Company, New York.

Gallant J. A. (1979).
Stringent control in *E. coli*.
Ann. Rev. Genet. **13**, 393-415.

Gates F. L. (1930).
A study of the bacterial action of ultraviolet light. III. The absorption of ultraviolet light by bacteria.
J. Gen. Physiol. **14**, 31-42.

Griego V. M., R. B. Webb and T. Matsushita (1981).
Dependence on stage of growth of mammalian cell sensitivity to near-UV irradiation.
Photochem. Photobiol. **33**, 211-214.

Grossman A. D., W. E. Taylor, Z. F. Burton, R. R. Burgess and C. A. Gross (1985).
Stringent response in *Escherichia coli* induces expression of heat shock proteins.
J. Mol. Biol. **186**, 357-365.

Haber F. and J. Weiss (1934).
The catalytic decomposition of hydrogen peroxide by iron salts.
Proc. Royal Soc. **147**, 332-351.

Halliwell B. and J. M. C. Gutteridge (1985).
Free Radicals in Biology and Medicine.
publ: Clarendon Press.

Harm W. (1980).
Biological effects of ultraviolet radiation.
publ: Cambridge University Press, London.

Harrison A. P. Jr. (1967).
Survival of bacteria. Harmful effects of light, with some comparisons with other adverse physical agents.
Ann. Rev. Microbiol. **21**, 143-156.

Hartman P. S. (1986).
In situ hydrogen peroxide production may account for a portion of NUV (300-400 nm) inactivation of stationary phase *Escherichia coli*.
Photochem. Photobiol. **43**, 87-89.

Hartman P. S. and A. Eisenstark (1978).
Synergistic killing of *E. coli* by near-UV radiation and hydrogen peroxide: Distinction between *recA*-repairable and *recA*-non-repairable damage.
J. Bacteriol. **133**, 769-774.

Hartman P. S., A. Eisenstark and P. G. Pauw (1979).
Inactivation of phage T7 by near-ultraviolet radiation plus hydrogen peroxide: DNA-protein crosslinks prevent DNA injection.
Proc. Natl. Acad. Sci. (USA). **76**, 3228-3232.

Hassan H. M. and I. Fridovich (1978).
Regulation of the synthesis of catalase and peroxidase in *Escherichia coli.*
J. Biol. Chem. **253** 6445-6450.

Hill R. F. (1956).
Effects of illumination on plaque formation by *Escherichia coli* infected with T1 bacteriophage.
J. Bacteriol. **71**, 231-235.

Hill R. F. and R. R. Feiner (1964).
Further studies on UV sensitive mutants of *E. coli* strain B.
J. Bacteriol. **35**, 105-114.

Hollaender A. (1943).
Effect of long ultraviolet and short visible radiation (3500 to 4900A) on *Escherichia coli.*
J. Bacteriol. **46**, 531-541.

Imlay J. A. and S. Linn (1986).
Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide.
J. Bacteriol. **166**, 519-527.

Ingraham J. L., O. Maaløe and F. C. Neidhardt (1983).
Growth of the bacterial cell.
publ: Sinauer Associates Inc. USA

Ito A. and T. Ito (1983).
Possible involvement of membrane damage in the inactivation by broad-band near-UV radiation in *Saccharomyces cerevisiae* cells.
Photochem. Photobiol. **37**, 395-401.

Jagger J. (1967).
Introduction to research to ultraviolet photobiology.
publ: Prentice Hall, New Jersey.

Jagger J. (1975).
Inhibition by sunlight of the growth of *Escherichia coli* B/r.
Photochem. Photobiol. **22**, 67-70.

Jagger J. (1985).
Solar-UV actions on living cells.
publ: Praeger Publishers, New York.

Jagger J., W. C. Wise and R. S. Stafford (1964).
Delay in growth and division induced by near-ultraviolet radiation in *Escherichia coli* B and its role in photoprotection and liquid holding recovery.
Photochem. Photobiol. **3**, 11-24.

Kelland L. R. (1984).
A study of near ultraviolet radiation induced damage in *Escherichia coli*.
Ph.D. Thesis. Univ. Bath, UK.

Kelland L. R., S. H. Moss and D. J. G. Davies (1983a).
An action spectrum for ultraviolet radiation induced membrane damage in *Escherichia coli* K12.
Photochem. Photobiol. **37**, 301-306.

Kelland L. R., S. H. Moss and D. J. G. Davies (1983b).
Recovery of *Escherichia coli* K-12 from near-ultraviolet radiation-induced membrane damage.
Photochem. Photobiol. **37**, 617-622.

Kelland L. R., S. H. Moss and D. J. G. Davies (1984a).
Leakage of $^{86}Rb^{+}$ after ultraviolet irradiation of *Escherichia coli* K-12.
Photochem. Photobiol. **39**, 329-335.

Kelland L. R., S. H. Moss and D. J. G. Davies (1984b).
The effect of the phase of growth on the sensitivity of four *Escherichia coli* DNA repair competent strains to near-ultraviolet light.
Photochem. Photobiol. **39**, 82s Abstract number THAM-H4.

Kelner A. (1949).
Effect of visible light on the recovery of *Streptomyces griseus* conidia from ultraviolet irradiation injury.
Proc. Natl. Acad. Sci. (USA). **35**, 73-79.

Klamer D. L. and R. W. Tuveson (1982).
The effect of membrane fatty acid composition on the near-UV (300-400 nm) sensitivity of *Escherichia coli* K1060.
Photochem. Photobiol. **35**, 167-173.

Kralli A. and S. H. Moss (1987).
The sensitivity of an actinic reticuloid cell strain to near-ultraviolet radiation and its modification by Trolox-C, a vitamin E analogue.
Brit. J. Dermatol. **116**, 761-772.

Kramer G. F. and B. N. Ames (1987).
Oxidative mechanisms of toxicity of low-intensity near-UV light in *Salmonella typhimurium*.
J. Bacteriol. **169**, 2259-2266.

Krueger J. H. and G. C. Walker (1984).
groEL and *dnaK* genes of *Escherichia coli* are induced by UV irradiation and naladixic acid in a *htpR*$^{+}$-dependent fashion.
Proc. Natl. Acad. Sci. (USA). **81**, 1499-1503.

Laffler T. and J. Gallant (1974)
spoT, a new genetic locus involved in the stringent response in *Escherichia coli*.
Cell. **1**, 27-30.

Lai L-W., J. M. Ducore and B. S. Rosenstein (1987).
DNA-protein crosslinking in normal human skin fibroblasts exposed to solar ultraviolet wavelengths.
Photochem. Photobiol. **46**, 143-146.

Lee P. C., B. R. Bochner and B. N. Ames (1983).
AppppA, heat-shock stress, and cell oxidation.
Proc. Natl. Acad. Sci. (USA). **80**, 7496-7500.

Ley R. D., B. A. Sedita and E. Boye (1978).
DNA polymerase-I mediated repair of 365 nm-induced single-strand breaks in the DNA of *Escherichia coli*.
Photochem. Photobiol. **27**, 323-327.

Loewen P. C. (1984).
Isolation of catalase-deficient *Escherichia coli* mutants and genetic mapping of *katE*, a locus that affects catalase activity.
J. Bacteriol. **157**, 622-626.

Loewen P. C. and B. L. Triggs (1984).
Genetic mapping of *katF* a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*.
J. Bacteriol. **160**, 668-675.

Loewen P. C., B. L. Triggs, C. S. George and B. E. Hrabarchuk (1985a).
Genetic mapping of *katG*, a locus that affects synthesis of the bifunctional catalase - peroxidase Hydroperoxidase I in *Escherichia coli*.
J. Bacteriol. **162**, 661-667.

Loewen P. C., J. Switala and B. L. Triggs-Raine (1985b).
Catalases HPI and HPII in *Escherichia coli* are induced independently.
Arch. Biochem. Biophys. **243**, 144-149.

Luckiesh M. (1946).
Applications of germicidal, erythemal and infrared energy.
publ: Van Nostrand, New York.

Mackay D. A., Eisenstark, R. B. Webb and M. S. Brown (1976).
Action spectra for lethality in recombination-less strains of *Salmonella typhimurium* and *Escherichia coli*.
Photochem. Photobiol. **24**, 337-343.

Mackay B. M. and C. M. Derrick (1982).
A comparison of solid and liquid media for measuring the sensitivity of heat-injured *Salmonella typhimurium* to selenite and tetrathionate media, and the time needed to recover resistance.
J. Appl. Bacteriol. **53**, 233-242.

Mackay B. M. and C. M. Derrick (1986a).
Elevation of the heat resistance of *Salmonella typhimurium* by sublethal heat shock.
J. Appl. Bacteriol. **61**, 389-393.

Mackay B. M. and C. M. Derrick (1986b).
Peroxide sensitivity of cold-shocked *Salmonella typhimurium* and *Escherichia coli* and its relationship to minimal medium recovery.
J. Appl. Bacteriol. **60**, 501-511.

Mackay B. M. and D. A. Seymour (1987).
The effect of catalase on recovery of heat-injured DNA-repair mutants of *Escherichia coli*.
J. Gen. Microbiol. **133**, 1601-1610.

Mathews M. M. and W. R. Sistrom (1959).
Function of carotenoid pigments in non-photosynthetic bacteria.
Nature (London). **184**, 1892-1893.

McCord J. M. and I. Fridovich (1968).
The reduction of cytochrome C by milk xanthine oxidase.
J. Biol. Chem. **243**, 5753-5760.

McCormick J. P. and A. Oczós (1979).
Biological effects of near-UV radiation 2'-acetylformanilide-sensitized formation of hydrogen peroxide from nucleosides and nucleotides.
Photochem. Photobiol. **29**, 1041-1044.

McCormick J. P., J. R. Fischer and J. P. Pachlatko (1976).
Characterization of a cell-lethal product from the photooxidation of tryptophan: Hydrogen peroxide.
Science. **191**, 468-469.

Miller J. H. (1972).
Experiments in molecular genetics.
publ: Cold Spring Harbor, New York.

Monod J. (1949).
The growth of bacterial cultures.
Ann. Rev. Microbiol. **3**, 371-394.

Morgan R. W., M. F. Christman, F. S. Jacobson, G. Storz and B. N. Ames (1986).
Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap.
Proc. Natl. Acad. Sci. (USA). **83**, 8059-8063.

Moss S. H. and K. C. Smith (1981).
Membrane damage can be a significant factor in the inactivation of *Escherichia coli* by near-ultraviolet radiation.
Photochem. Photobiol. **33**, 203-210.

Neidhardt F. C., R. A. Van Bogelen and V. Vaughn (1984).
The genetics and regulation of heat-shock proteins.
Ann. Rev. Genet. **18**, 295-329.

Nies D. and H. G. Schlegel (1984).
Use of catalase from *Escherichia coli* in model experiments for oxygen supply of microorganisms with hydrogen peroxide.
Biotechnol. Bioeng. **26**, 737-741.

Novick A. (1955).
Growth of bacteria.
Ann. Rev. Microbiol. **9**, 97-110.

Ossanna N., K. P. Peterson and D. W. Mount (1987).
UV-inducible SOS response in *Escherichia coli*.
Photochem. Photobiol. **45**, 905-908.

Peak M. J. (1970).
Some observations on the lethal effects of near-ultraviolet light on *Escherichia coli* compared with the lethal effects of far-ultraviolet light.
Photochem. Photobiol. **12**, 1-8.

Peak M. J. and J. G. Peak (1975).
Protection by AET against inactivation of transforming DNA by near-ultraviolet light: Action spectrum.
Photochem. Photobiol. **22**, 147-148.

Peak M. J. and J. G. Peak (1982).
Single-strand breaks induced in *Bacillus subtilis* by ultraviolet light: action spectrum and properties.
Photochem. Photobiol. **35**, 675-680.

Peak M. J., J. G. Peak and R. B. Webb (1973).
Inactivation of transforming DNA by ultraviolet light. I. Near-UV action spectrum for marker inactivation. II. Protection by histidine against near-UV irradiation: Action spectrum. III. Further observations on the effects of 365 nm radiation.
Mutat. Res. **20**, 129-148.

Peak J. G., C. S. Foote and M. J. Peak (1981).
Protection by DABCO against inactivation of transforming DNA by near-ultraviolet light: action spectra and implications for involvement of singlet oxygen.
Photochem. Photobiol. **34**, 45-49.

Peak J. G., M. J. Peak and M. MacCoss (1984).
DNA breakage caused by 334 nm ultraviolet light is enhanced by naturally occuring nucleic acid components and nucleotide coenzymes.
Photochem. Photobiol. **39**, 713-716.

Peters J. (1977).
In vivo photoinactivation of *Escherichia coli* ribonucleotide reductase by near-ultraviolet light.
Nature. (London) **267**, 546-548.

Peters J. and J. Jagger (1981).
Inducible repair of near-UV radiation lethal damage in *E. coli*.
Nature. (London) **289**, 194-195.

Pizarro R. A. and L. V. Orce (1988).
Membrane damage and recovery associated with growth delay induced by near-UV radiation in *Escherichia coli* K12.
Photochem. Photobiol. **47**, 391-397.

Privalle C. T. and I. Fridovich (1987).
Induction of superoxide dismutase in *Escherichia coli* by heat shock.
Proc. Natl. Acad. Sci. (USA). **84** 2723-2726.

Pryor W. A. (1976).
Free Radicals in Biology.
ed: W. A. Pryor
publ: Academic Press
vol: 1-5.

Ramabhadran T. V. (1975).
Effects of near-ultraviolet and violet radiations (313-405 nm) on DNA, RNA and protein synthesis in *E. coli* B/r: Implications for growth delay.
Photochem. Photobiol. **22**, 117-123.

Ramabhadran T. V. and J. Jagger (1975).
Evidence against DNA as the target for 334 nm-induced growth delay in *Escherichia coli.*
Photochem. Photobiol. **21**, 227-233.

Ramabhadran T. V. and J. Jagger (1976).
Mechanism of growth delay induced in *Escherichia coli* by near ultraviolet radiation.
Proc. Natl. Acad. Sci. (USA). **73**, 59-63.

Ramabhadran T. V., T. Fossum and J. Jagger (1976).
In vivo induction of 4-thiouridine-cytidine adducts in tRNA of *E. coli* B/r by near-ultraviolet radiation.
Photochem. Photobiol. **23**, 315-321.

Rayman M. K., B. Aris and H. B. El Derea (1978).
The effect of compounds which degrade hydrogen peroxide on the enumeration of heat-stressed cells of *Salmonella senftenberg.*
Can. J. Microbiol. **24**, 883-885.

Richter H. E. and P. C. Loewen (1981).
Induction of catalase in *Escherichia coli* by ascorbic acid involves hydrogen peroxide.
Biochem. Biophys. Res. Comm. **100**, 1039-1046.

Rosenstein B. S. and J. M. Ducore (1983).
Induction of DNA strand breaks in normal human fibroblasts exposed to monochromatic ultraviolet and visible wavelengths in the 240-546 nm range.
Photochem. Photobiol. **38**, 51-55.

Ruis H. (1979).
The biosynthesis of catalase.
Can. J. Biochem. **57**, 1122-1130.

Rupert C. S. (1960).
Photoreactivation of transforming DNA by an enzyme from baker's yeast.
J. Gen. Physiol. **43**, 573-595.

Rupert C. S., S. H. Goodgal and R. M. Herriott (1958).
Photoreactivation *in vitro* of ultraviolet inactivated *Hemophilus influenzae* transforming factor.
J. Gen. Physiol. **41**, 451-471.

Rupp W. D. and P. Howard-Flanders (1968).
Discontinuities in the DNA synthesized in an excision deficient strain of *Escherichia coli* following ultraviolet radiation.
J. Mol. Biol. **31**, 291-304.

Sah N. K. and P. C. Kesavan (1987).
Post-irradiation modification of oxygen-dependent and independent damage by catalase in barley seeds.
Int. J. Radiat. Biol. **51**, 665-672.

Sammartano L. J. and R. W. Tuveson (1983).
Escherichia coli xthA mutants are sensitive to inactivation by broad spectrum near-UV (300-400 nm) radiation.
J. Bacteriol. **156**, 904-906.

Sammartano L. J. and R. W. Tuveson (1984).
The effects of exogenous catalase on broad-spectrum near-UV (300-400 nm) treated *Escherichia coli* cells.
Photochem. Photobiol. **40**, 607-612.

Sammartano L. J. and R. W. Tuveson (1985).
Hydrogen peroxide induced resistance to broad-spectrum near-ultraviolet light (300-400 nm) inactivation in *Escherichia coli*.
Photochem. Photobiol. **41**, 367-370.

Sammartano L. J., R. W. Tuveson and R. Davenport (1986).
Control of sensitivity to inactivation by H_2O_2 and broad-spectrum near-UV radiation by the *Escherichia coli katF* locus.
J. Bacteriol. **168**, 13-21.

Schonbaum G. and B. Chance (1976).
in: *The Enzymes*
ed: P. D. Boyer
publ: Academic Press
3rd ed. Vol. 13, pp. 363-408.

Sedgwick S. G. (1986).
Inducible DNA repair in microbes.
Microbiological Sciences. **3**, 76-83.

Setlow R. B. and W. L. Carrier (1964).
The disappearance of thymine dimers from DNA, an error correcting mechanism.
Proc. Natl. Acad. Sci. (USA). **51**, 226-231.

Smith K. C. (1976).
The radiation-induced addition of proteins and other molecules to nucleic acids.
in: *Photochemistry and Photobiology of Nucleic Acids*.
ed: S. Y. Wang
publ: Academic Press, New York
Vol. II, pp. 187-218.

Smith K. C., T-C. V. Wang and R. C. Sharma (1987).
recA-dependent DNA repair in UV-irradiated *Escherichia coli*.
J. Photochem. Photobiol. B: Biology. **1**, 1-11.

Stephens J. C., S. W. Artz and B. N. Ames (1975).
Guanosine T'-diphosphate 3'-diphosphate (ppGpp): a positive effector for histidine operon transcription and a general signal for amino acid deficiency.
Proc. Natl. Acad. Sci. (USA). **72**, 4389-4393.

Sun A. S., B. B. Aggawal and L. Packer (1975).
Enzyme levels of normal human cells: Aging in culture.
Arch. Biochem. Biophys. **170**, 1-11.

Sutherland B. M. (1977).
Symposium on molecular mechanisms in photoreactivation: Fundamentals of photoreactivation.
Photochem. Photobiol. **25**, 413-414.

Sutherland J. C. and K. P. Griffin (1981).
Absorption spectrum of DNA for wavelengths greater than 300 nm.
Radiat. Res. **86**, 399-409.

Thiam K. and A. Favre (1984).
Role of the stringent response in the expression and mechanism of near-ultraviolet induced growth delay.
Eur. J. Biochem. **145**, 137-142.

Thomas G. and A. Favre (1975).
4-thiouridine as the target for near-ultraviolet light induced growth delay in *Escherichia coli*.
Biomedical Biophysical Res. Comm. **66**, 1454-1461.

Thomas G., K. Thiam and A. Favre (1981).
Transfer RNA thiolated pyrimidines as targets for near-UV-induced synthesis of guanosine tetraphosphate in *Escherichia coli*.
Eur. J. Biochem. **119**, 381-388.

Tsai S-C. and J. Jagger (1981).
The roles of the *rel*$^+$ gene and of 4-thiouridine in killing and photoprotection of *Escherichia coli* by near-ultraviolet radiation.
Photochem. Photobiol. **33**, 825-834.

Tuveson R. W. (1980).
Genetic control of near-UV sensitivity independent of excision deficiency (*uvrA6*) in *Escherichia coli* K12.
Photochem. Photobiol. **32**, 703-705.

Tuveson R. W. (1981).
The interaction of a gene (*nur*) controlling near-UV sensitivity and the *polA1* gene in strains of *E. coli* K12.
Photochem. Photobiol. **33**, 919-923.

Tuveson R. W. and R. B. Jonas (1979).
Genetic Control of near-UV (300-400 nm) sensitivity independent of the *recA* gene in strains of *Escherichia coli* K12.
Photochem. Photobiol. **30**, 667-676.

Van Sluys M-A., R. Alcantara-Gomes and C. F. M. Menck (1986).
Escherichia coli xthA mutant is not hypersensitive to ascorbic acid/copper treatment - an H_2O_2 generating reaction.
Mutat. Res. **174**, 265-269.

Wagner S., W. D. Taylor, A. Keith and W. Snipes (1980).
Effects of acridine plus near ultraviolet light on *Escherichia coli* membranes and DNA *in vivo*.
Photochem. Photobiol. **32**, 771-779.

Walker G. C. (1985).
Inducible DNA repair systems.
Ann. Rev. Biochem. **54**, 425-457.

Walrant P. and R. Santus (1974).
N-formyl-kynurenine, a tryptophan photooxidation product, as a photodynamic sensitizer.
Photochem. Photobiol. **19**, 411-417.

Wanner B. L. (1986).
Novel regulatory mutants of the phosphate regulon in *Escherichia coli* K-12.
J. Mol. Biol. **191**, 39-58.

Webb R. B. (1977).
Lethal and mutagenic effects of near-ultraviolet radiation.
Photochem. Photobiol. Revs. 2, 169-261.
publ: Plenum Press.
ed: K. C. Smith.

Webb R. B. and M. S. Brown (1976).
Sensitivity of strains of *Escherichia coli* differing in repair capability to far-UV, near-UV and visible radiations.
Photochem. Photobiol. **24**, 425-432.

Webb R. B. and J. R. Lorenz (1977).
Oxygen dependence and repair of lethal effects of near-ultraviolet and visible light.
Photochem. Photobiol. **12**, 283-289.

Webb R. B., M. S. Brown and R. M. Tyrrell (1976).
Lethal effects of pyrimidine dimers induced at 365 nm in strains of *E. coli* differing in repair capability.
Mutat. Res. **37**, 163-172.

Webb R. B., R. M. Tyrrell and M. S. Brown (1978).
Synergism between 365 and 254-nm radiations for inactivation of *Escherichia coli*.
Radiat. Res. **74**, 298-311.

Werbin H. (1977).
DNA photolyase.
Photochem. Photobiol. **26**, 675-678.

Winquist L., U. Rannug, A. Rannug and C. Ramel (1984).
Protection from toxic and mutagenic effects of H_2O_2 by catalase induction in *Salmonella typhimurium*.
Mutat. Res. **141**, 145-147.

Yoakum G. H. (1975).
Tryptophan photoproduct(s): Sensitized induction of strand breaks (or alkali-labile bonds) in bacterial deoxyribonucleic acid during near-ultraviolet irradiation.
J. Bacteriol. **122**, 199-205.

Yoakum G. and A. Eisenstark (1972).
Toxicity of L-tryptophan photoproduct on recombinationless (*rec*) mutants of *Salmonella typhimurium*.
J. Bacteriol. **112**, 653-655.

Yoakum G., W. Ferron, A. Eisenstark and R. B. Webb (1974).
Inhibition of replication gap closure in *Escherichia coli* near-ultraviolet light photoproducts of L-tryptophan.
J. Bacteriol. **119**, 62-69.

Yoshpe-Purer Y. and Y. Henis (1976).
Factors affecting catalase levels and sensitivity to hydrogen peroxide in *Escherichia coli*.
Appl. Environ. Microbiol. **32**, 465-469.

Yoshpe-Purer Y., Y. Henis and J. Yashphe (1977).
Regulation of catalase level in *Escherichia coli* K12.
Can. J. Microbiol. **23**, 84-91.

A Few mistakes

p2
23.
27
93.
157.

p1 - E. coli exposed to UV?
15 - no plates anaerobically?
- p18. -
42 - γ-radiation effects mainly genetic?
may be indirect rather than direct?

79 - different shapes
93 - Did you show time 0·30 not important?
96 - why not regular for longer
105 - why is UVR resistant
124 - how much new, how much confirming data already known?
→ assumes damage to DNA? what about membrane

p140 - K12 has large nutrient pools B/r not. (N.15+)
mixed sensitivity of B/r may be due to incomplete cell wall
→ show difference in genome component
p144 - no sensitivity because with low number there is enough nutrient material to form membrane
p135 can't see shape but might expect more in a shoulder as in K53

with SR395 (K12) same as B/r. Kodak.
p156.

p160 - why was this done?
* 207 - why not follow this up - what about membrane?
How would you do it - what about leakage.